Coronary Drugs

Michael Gralinski, Liomar A. A. Neves, and Olga Tiniakova

Contents

M. Gralinski (✉) • L.A.A. Neves • O. Tiniakova
CorDynamics, Inc., Chicago, IL, USA
e-mail: mrg@cordynamics.com; neves@cordynamics.com; opt@cordynamics.com

Isolated Organs

Heart–Lung Preparation

Purpose and Rationale

The isolated heart–lung of the dog was introduced by Knowlton and Starling (1912). Since then, the dog model has been used for many physiological and pharmacological studies (Krayer 1931; Krayer and Mendez 1942; Somani and Blum 1966; Takeda et al. 1973; Ishikawa et al. 1978, 1983; Ono and O'Hara 1984; Ono et al. 1984; Caffrey et al. 1986; Hausknecht et al. 1986; Fessler et al. 1988; Seifen et al. 1987, 1988; Naka et al. 1989). More recently, the rat model has been preferred (Dietz 1984, 1987; Onwochei et al. 1987; Onwochei and Rapp 1988; Kashimoto et al. 1987, 1990, 1994, 1995; Fukuse et al. 1995).

Procedure

Wistar rats weighing 300–320 g are anesthetized with 50 mg/kg pentobarbital i.p. Tracheotomy is performed and intermittent positive pressure ventilation is instituted with air. The chest is opened and flooded with ice-cold saline and the heart arrested. Cannulae are inserted into the aorta and the superior (for measurement of central venous pressure) and inferior venae cavae. The heart–lung preparation is perfused with a solution containing rat blood cells from another rat and Krebs–Ringer bicarbonate buffer, with hematocrit and pH of 25 % and 7.4, respectively. The

F.J. Hock (ed.), *Drug Discovery and Evaluation: Pharmacological Assays*,
DOI 10.1007/978-3-319-05392-9_5

concentrations of the buffer constituents (mM) are NaCl 127, KCl 5.1, $CaCl_2$ 2.2, KH_2PO_4 1.3, $MgSO_4$ 2.6, $NaHCO_3$ 15, glucose 5.5, and heparin. The perfusate pumped from the aorta passes through a pneumatic resistance and is collected in a reservoir maintained at 37 °C and then returned to the inferior vena cava. In this model, no other organs except the heart and lung are perfused. Cardiac output is determined by the inflow as long as the heart does not fail. Mean arterial pressure is regulated by the pneumatic resistance. Heart rate is recorded by a bioelectric amplifier, and cardiac output is measured with an electromagnetic blood flow meter. Arterial pressure and right atrial pressure are measured with transducers and amplifiers. The heart is perfused initially with cardiac output of 30 ml/min and mean arterial pressure of 80 mmHg. Test drugs are administered into the perfusate 5 min after start of the experiment.

Evaluation

Hemodynamic data within groups are analyzed by two-way analysis of variance (ANOVA) with repeated measures. Recovery time is measured by the Kruskal–Wallis test. The other data are analyzed by one-way ANOVA followed by the Dunnett test for multiple comparisons.

Modifications of the Method

Using the Starling heart–lung preparation in dogs, Wollenberger (1947) studied the energy-rich phosphate supply of the failing heart.

Shigei and Hashimoto (1960) studied the mechanism of the heart failure induced by pentobarbital, quinine, fluoroacetate, and dinitrophenol in dog's heart–lung preparation and effects of sympathomimetic amines and ouabain on it.

Imai et al. (1961) used heart–lung preparations of the dog to study the cardiac actions of methoxamine with special reference to its antagonistic action to epinephrine.

Capri and Oliverio (1965) and Beaconsfield et al. (1974) used the heart–lung preparation of the **guinea pig**.

Robicsek et al. (1985) studied the metabolism and function of an autoperfused heart–lung preparation of the **dog**.

The **dog heart–lung preparation** was used:

By Seifen et al. (1988) to study the interaction of a calcium channel agonist with the effects of digoxin

By Somani and Blum (1966) to study blockade of epinephrine- and ouabain-induced cardiac arrhythmias in the dog

By Riveron et al. (1988) to investigate the energy expenditure of an autoperfusing heart–lung preparation

By Namakura et al. (1987) to study the role of pulmonary innervation in an in situ lung-perfusion preparation as a new model of neurogenic pulmonary edema

By Hausknecht et al. (1986) to investigate the effects of lung inflation on blood flow during cardiopulmonary resuscitation

By Caffrey et al. (1986) to evaluate the effect of naloxone on myocardial responses to isoproterenol

By Ono et al. (1984) to estimate the cardiodepressant potency of various beta-blocking agents

By Ishikawa et al. (1983) for a graphical analysis of drug effects in the dog heart–lung preparation – with particular reference to the pulmonary circulation and effects of norepinephrine and 5-hydroxytryptamine

By Iizuka (1983) to study the cardiac effects of acetylcholine and its congeners

By Fessler et al. (988) to investigate the mechanism of reduced LV afterload by systolic and diastolic positive pleural pressure

By Takeda et al. (1973) to study the cardiac actions of oxprenolol

Beaconsfield et al. (1974) used the heart–lung preparation of **guinea pigs** to study the cardiac effect of delta-9-tetrahydrocannabinol.

The **rabbit** autoperfusing heart–lung preparation was used by Muskett et al. (1986, 1988).

The isolated heart–lung preparation in the **cat** was described by Beaufort et al. (1993).

Kontos et al. (1987, 1988) harvested heart–lung blocks from **calves**.

Kissling et al. (2000) described a modified heart–lung preparation of the **rat** to allow the

systolic coronary flow to be distinguished from diastolic flow and to permit the effect of coronary compliance on coronary circulation to be studied.

References and Further Reading

Beaconsfield P, Oakley C, Carpi A, Rainsbury R, del Basso P (1974) Cardiac effect of delta-9-tetrahydrocannabinol on a heart-lung preparation and on the intact animal. Eur J Cardiol 2:167–173

Beaufort AM, Wierda JMKH, Houwertjes MC, Kleef UW, Meijer DKF (1993) The isolated heart-lung preparation in the cat. An *in situ* model to study the role of the lungs in the disposition of drugs. J Pharmacol Toxicol Methods 29:147–156

Caffrey JL, Wooldridge CB, Gaugl JF (1986) Naloxone enhances myocardial responses to isoproterenol in dog isolated heart-lung. Am J Physiol 250(Heart Circ Physiol 19):H749–H754

Capri A, Oliverio A (1965) Effect of reserpine on the heart-lung preparation of guinea pig. Arch Int Pharmacodyn Ther 157:470–486

Dietz JR (1984) Release of natriuretic factor from rat heart-lung preparation by atrial distension. Am J Physiol 247(Regulatory Integrative Comp Physiol 16):R1093–R1096

Dietz JR (1987) Control of atrial natriuretic factor release from a rat heart-lung preparation. Am J Physiol 252(Regulatory Integrative Comp Physiol 21):R498–R502

Fessler HE, Brower RG, Wise RA, Permutt S (1988) Mechanism of reduced LV afterload by systolic and diastolic positive pleural pressure. J Appl Physiol 65:1244–1250

Fukuse T, Albes JM, Takahashi Y, Brandes H, Hausen B, Schäfers HJ (1995) Influence of red blood cells in an ex vivo rat heart-lung model. J Surg Res 59:399–404

Hausknecht MJ, Wise RA, Brower RG, Hassapoyannes C, Weisfeldt ML, Suzuki J, Permutt S (1986) Effects of lung inflation on blood flow during cardiopulmonary resuscitation in the canine isolated heart-lung preparation. Circ Res 59:676–683

Iizuka H (1983) Cardiac effects of acetylcholine and its congeners as assessed in canine heart-lung preparation. Folia Pharmacol Jpn 81:441–449

Imai S, Shigei T, Hashimoto K (1961) Cardiac actions of methoxamine with special reference to its antagonistic action to epinephrine. Circ Res 9:552–560

Ishikawa N, Taki K, Hojo Y, Hagino Y, Shigei T (1978) Direct recording of cardiac output- and venous return-curves in the dog heart-lung preparation for a graphical analysis of the effects of cardioactive drugs. Jpn Heart J 19:775–782

Ishikawa N, Taki K, Hojo Y, Hagino Y, Shigei T (1983) Graphical analysis of drug effects in the dog heart-lung preparation – with particular reference to the pulmonary circulation and effects of norepinephrine and 5-hydroxytryptamine. Jpn J Pharmacol 33:785–794

Jerusalem E, Starling EH (1910) On the significance of carbon dioxide for the heart beat. J Physiol 40:279–294

Kashimoto S, Tsuji Y, Kumazawa T (1987) Effects of halothane and enflurane on myocardial metabolism during postischaemic reperfusion in the rat. Acta Anaesth Scand 31:44–47

Kashimoto S, Kume M, Kumazawa T (1990) Functional and metabolic effects of bupivacaine and lignocaine in the rat heart-lung preparation. Br J Anaesth 65:521–526

Kashimoto S, Nakamura T, Kume M, Nonaka A, Kumazawa T (1994) Effects and interaction of nicardipine and volatile anesthetics in the rat heart-lung preparation. J Anesth 8:78–83

Kashimoto S, Namakura T, Furuya A, Kume M, Kumazawa T (1995) Alteration of cardiac function and metabolism in the rat heart-lung preparation by methyl methacrylate and their protection by ulinastatin. Jpn J Anesth 44:1477–1481

Kissling G, Blickle B, Pascht U (2000) Modified heart-lung preparation for the evaluation of systolic and diastolic coronary flow in rats. Am J Physiol Heart Circ Physiol 278(1): H277–H284

Knowlton FP, Starling EH (1912) The influence of variations in temperature and blood pressure on the performance of the isolated mammalian heart. J Physiol 44:206–219

Kontos GJ Jr, Borkon AM, Adachi H, Baumgartner WA, Hutchins GM, Brawn J, Reitz BA (1987) Successful extended cardiopulmonary preservation in the autoperfused working heart-lung preparation. Surgery 102:269–276

Kontos GJ Jr, Borkon AM, Baumgartner WA, Fonger JD, Hutchins GM, Adachi H, Galloway E, Reitz BA (1988) Improved myocardial and pulmonary preservation by metabolic substrate enhancement in the autoperfused working heart-lung preparation. J Heart Transplant 7:140–144

Krayer O (1931) Versuche am isolierten Herzen. Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 162:1–28

Krayer O, Mendez R (1942) Studies on veratrum alkaloids. I. The action of veratrine upon the isolated mammalian heart. J Pharmacol Exper Ther 74:350–364

Merin RG (1988) The isolated heart preparation. Br J Anesth 60:28S–34S

Muskett AD, Burton NA, Gay WA, Miller M, Rabkin MS (1986) Preservation in the rabbit autoperfusing heart-lung preparation: a potential role of indomethacin. Surg Forum 37:252–254

Muskett AD, Burton NA, Grossman M, Gay WA Jr (1988) The rabbit autoperfusing heart-lung preparation. J Surg Res 44:104–108

Naka Y, Hirose H, Matsuda H, Nakano S, Shirakura R, Kawaguchi A, Miyamoto Y, Miyagawa S, Fukushima N, Kawashima Y (1989) Prevention of pulmonary edema in autoperfusing heart-lung preparations by FUT-175 and leukocyte depletion. Transplant Proc 21:1353–1356

Namakura J, Zhang S, Ishikawa N (1987) Role of pulmonary innervation in canine *in situ* lung-perfusion preparation: a new model of neurogenic pulmonary edema. Clin Exp Pharmacol Physiol 14:535–543

Ono H, Kanazawa Y, O'Hara N, Hashimoto K (1984) Estimation of cardiodepressant potency of nadolol, alprenololol, propranolol and pindolol, β-blocking agents, in heart-lung preparation and blood-perfused excised papillary muscle preparation of the dog. Jpn J Pharmacol 36:507–517

Ono H, O'Hara N (1984) A study of the cardiodepressant action of a β-blocking agent carteolol in heart-lung preparation of the dog. Jpn Circ J 86:1030–1044

Onwochei MO, Rapp JP (1988) Biochemically stimulated release of atrial natriuretic factor from heart-lung preparation in Dahl rats. Proc Soc Exp Biol Med 188:395–404

Onwochei MO, Snajdar RM, Rapp JP (1987) Release of atrial natriuretic factor from heart-lung preparations of inbred Dahl rats. Am J Physiol 253(Heart Circ Physiol 22): H1044–H1052

Riveron FA, Ross JH, Schwartz KA, Casey G, Sanders ON, Eisiminger R, Magilligan DJ Jr (1988) Energy expenditure of autoperfusing heart-lung preparation. Circulation 78:II Suppl III-103–III-109

Robicsek F, Masters TN, Duncan GD, Denyer MH, Rise HE, Etchison M (1985) An autoperfused heart-lung preparation: metabolism and function. Heart Transplant 4:334–338

Seifen E, Kennedy RH, Seifen AB (1988) Interaction of BAY K-8644 with effects of digoxin in the dog heart-lung preparation. Eur J Pharmacol 158:109–117

Seifen E, Seifen AB, Kennedy RH, Bushman GA, Loss GE, Williams TG (1987) Comparison of cardiac effects of enflurane, isoflurane, and halothane in the dog heart-lung preparation. J Cardiothorac Anesth 1:543–553

Shigei T, Hashimoto K (1960) Study on the mechanism of the heart failure induced by pentobarbital, quinine, fluoroacetate and dinitrophenol in dog's heart-lung preparation and effects of sympathomimetic amines and ouabain on it. Jpn J Pharmacol 9:109–122

Somani P, Blum BK (1966) Blockade of epinephrine- and ouabain-induced cardiac arrhythmias in the dog heart-lung preparation. J Pharmacol Exp Ther 152:235–242

Takeda K, Iizuka K, Imai S (1973) Cardiac actions of oxprenolol as studied in dog heart-lung preparations. Arzneim Forsch/Drug Res 23:1446–1450

Wollenberger A (1947) On the energy-rich phosphate supply of the failing heart. Am J Physiol 150:733–745

Isolated Heart According to Langendorff

Purpose and Rationale

More than 100 years ago, Langendorff (1895) described studies on isolated surviving mammalian hearts using mainly cats as donors. Since then, the method has been improved from the technical site and is nowadays used for studies with guinea pig, rabbit, or rat hearts. In principle, the heart is perfused in retrograde direction from the aorta either at constant pressure or at constant flow with oxygenated saline solutions. Retrograde perfusion closes the aortic valves, just as in the in situ heart during diastole. The perfusate is displaced through the coronary arteries flowing off the coronary sinus and the opened right atrium. In this original setup, the ventricles do not fill with perfusate and therefore do not perform pressure-volume work. Parameters usually measured are contractile force, coronary flow, and cardiac rhythm.

Procedure

Guinea pigs of either sex weighing 300–500 g are sacrificed by stunning. For studies of biochemical parameters in tissue and perfusate, removal of the heart during barbiturate anesthesia and artificial respiration is recommended. The heart is removed as quickly as possible and placed in a dish containing Ringer's solution at 37 °C. Associated pericardial and lung tissues are removed. The aorta is located and cut just below the point of its division. A glass or plastic cannula is introduced into the aorta and tied with two threads, and perfusion is started with oxygenated Ringer's solution or Krebs–Henseleit buffer. The heart is transferred to a double-walled Plexiglass perfusion apparatus which is kept at 37 °C by the water from a thermostat. Oxygenated Ringer's solution is perfused at a constant pressure of 40 mmHg and at a temperature of 37 °C from a reservoir. A small steel hook with a string is attached to the apex of the heart. Contractile force is measured isometrically by a force transducer with a preload of 2.5 g and recorded on a polygraph. Coronary flow is measured by a drop counter. Alternatively, flow measurements can be performed using a mechanic-electronic flow meter consisting of a vertical pipe and a magnetic valve (Hugo Sachs Electronic KG, Germany). Heart rate is measured through a chronometer coupled to the polygraph. Drugs are injected into the perfusion medium just above the aortic cannula.

Critical Assessment of the Method

A reappraisal of the Langendorff heart preparation was given by Broadley (1979) underlining the usefulness to test coronary vasodilating drugs. The value of the Langendorff method can be best assessed by demonstrating a few of its applications in physiology and pharmacology. Direct effects can be measured as well as the antagonism against various physiological and pharmacological agents.

Modifications of the Method

A survey on various modifications of the Langendorff technique and the **isolated working heart preparation** has been given by Ross (1972).

Neely et al. (1967) inserted a second cannula into a pulmonary vein or the left atrium. Perfusate from a reservoir flows via this cannula through the mitral valve into the left ventricle. During the systole of the heart, the left ventricle repumps the perfusate through the aorta into the reservoir. The perfusate flowing through the coronary arteries and dripping off from the outside of the heart is collected in a vessel below the heart and recirculated into the reservoir with a roller pump.

Flynn et al. (1978) underlined the difference of this working heart preparation to the original Langendorff method and reported the effects of histamine and noradrenaline on peak left ventricular systolic pressure, contractility, sinus rate, coronary flow, aortic flow, total cardiac output, and external pressure-volume work. Therefore, this method is reported separately.

Ishiu et al. (1996) measured simultaneously Ca^{2+}-dependent indo-1 fluorescence and left ventricular pressure on a beat-to-beat basis in Langendorff guinea pig hearts and investigated the changes in Ca^{+2} transient and left ventricular function during positive inotropic stimulation and myocardial ischemia.

Hukovic and Muscholl (1962) described the preparation of the isolated **rabbit** heart with intact sympathetic nervous supply from the right stellate ganglion.

Hendrikx et al. (1994) used the isolated perfused rabbit heart to test the effects of an Na^+/H^+ exchange inhibitor on postischemic function, resynthesis of high-energy phosphate, and reduction of Ca^{2+} overload.

Michio et al. (1985) modified the Langendorff method in rabbits to a working heart preparation by cannulating the left atrium. At a pressure of 20 cm H_2O in the left atrium, the heart pumped the solution against a hydrostatic pressure of 100 cm H_2O. Aortic flow, systolic aortic pressure, coronary flow, and heart rate were measured.

The influence of an ACE-inhibitor on heart rate, lactate in the coronary effluate, and GTP level in the myocardium after 60 min hypothermic cardiac arrest was studied in working heart preparation of rabbits by Zegner et al. (1996).

Gottlieb and Magnus (1904) introduced the so-called balloon method. A small balloon fixed to the tip of a catheter is filled with water and inserted into the left ventricle via one of the pulmonary veins, the left atrium and the mitral valve. The balloon size has to fit the volume of the left ventricle, and therefore, its size depends on the animal species and body weight. The catheter can be fixed by tying the pulmonary vein stems. Via a three-way valve, the balloon can be extended to a given preload. The beating heart now exerts a rhythmic force to the balloon and thus to the membrane of a pressure transducer. The advantages of this method are that force development and preload can be stated reproducibly in pressure units [mmHg], left ventricular contraction curves can be used for further calculations, and continuous heart rate recordings can be carried out without any problems when using a rate meter.

Sakai et al. (1983) reported a similar method adapted to **mice**.

Bardenheuer and Schrader (1983) described a method whereby the balloon is inserted into the left ventricle as described above. However, isovolumetric pressure in the left ventricle is not measured. Instead, the fluid in the balloon is pumped through the cannula into a closed extracorporeal circulation. The fluid is forced into one direction by two recoil valves. The balloon is made of silicone material using a Teflon form (Linz et al. 1986). The dimensions of the form are derived from casts of the left ventricle of K^+-arrested heart by injection of dental cement (Palavit 55, Kulzer and Co, GmbH, Germany). During each heart beat, the fluid volume expelled from the balloon corresponding to the stroke volume of the heart can be recorded by means of a flow meter probe and an integrator connected in series. Preload and afterload can be adjusted independently from each other. The perfusate flow (retrograde into the aorta and through the coronary arteries) is recorded separately.

The following parameters were measured in isolated **rat** hearts (Linz et al. 1986, 1990):

- LVP (left ventricular pressure) with Statham pressure transducer P 23DB, which on differentiation yielded LV dp/dt_{max} and HR (heart rate). Cardiac output and coronary flow (CF) are determined by electromagnetic flow probes in the outflow system and in the aortic cannula, respectively. Coronary venous pO_2 is measured with a catheter placed in the pulmonary artery by a type E 5046 electrode connected to a PMH 73 pH/blood gas monitor (radiometer). An epicardial electrocardiogram recording is obtained via two silver electrodes attached to the heart. All parameters are recorded on a Brush 2600 recorder.
- Myocardial oxygen consumption (MWO_2) [ml/min/g wet weight] is calculated according to the equation

$$MVO_2 = CF \times (P_a = P_V) \times (c/760) \times 100$$

where CF is the coronary flow [ml/min/g], P_a is the oxygen partial pressure of arterial perfusate (650 mmHg), P_v is the oxygen partial pressure of the venous effluent perfusate [mmHg], and c is the 0.0227 ml O_2/ml perfusate representing the Bunsen solubility coefficient of oxygen dissolved in perfusate at 37 °C (Zander and Euler 1976).

For the determination of lactate dehydrogenase (LDH) and creatine kinase (CK) activities in

the perfusate, samples are taken from the coronary effluent.

After the experiments, hearts are rapidly frozen in liquid nitrogen and stored at –80 °C. Of the left ventricle, 500 mg are taken, put into 5 ml ice-cold $HClO_4$, and disrupted with an ULTRA-TURRAX (Junke and Kunkel, Ika-Werk, Type TP). Glycogen is hydrolyzed with amyloglucosidase (pH 4.8) and determined as glucose. Furthermore, ATP and creatine phosphate are measured.

Avkiran and Curtis (1991) constructed a dual lumen aortic cannula which permits independent perfusion of left and right coronary beds in isolated rat hearts without necessitating the cannulation of individual arteries.

Igic (1996) described a modification of the isolated perfused working rat heart. A special double cannula was designed consisting of an outer cannula that is inserted into the aorta and an inner cannula that is advanced into the left ventricle. The perfusion fluid flows through the inner cannula into the left ventricle and is ejected from there into the aorta. If the outer cannula system is closed, the fluid perfuses the coronary vessels and drips off outside the heart. When the outer cannula is open and certain pressure resistance is applied, a fraction of the ejected fluid perfuses coronary vessels and the rest is expelled. Because the inner cannula can be easily retracted into the outer cannula, which is placed in the aorta, the preparation provides an opportunity to use the same heart as a "working" or "nonworking" model for investigating functions of the heart.

By labeling glucose, lactate, or fatty acids in the perfusate with ^{3}H or ^{14}C, Barr and Lopaschuk (1997) directly measured energy metabolism in the isolated rat heart.

Krzeminski et al. (1991) described a new concept of the isolated heart preparation with online computerized data evaluation. Left ventricular pressure was recorded by means of a balloon catheter, while special suction electrodes obtained the high-amplitude, noise-free electrogram recordings. The coronary effluent partial pressure of oxygen was continuously monitored, which enabled the calculation of myocardial oxygen consumption (MVO_2). The effluent partial pressure of carbon dioxide and pH value were also measured simultaneously. A computerized system of data acquisition, calculation, storage, and end report was described.

Döring (1990) described continuous simultaneous **ultrasonic recording** of two cardiac diameters in an isolated perfused guinea pig heart. For the measurement of the left ventricular transversal diameter, the ultrasonic transmitter was positioned at the epicardium at the largest cardiac diameter. The corresponding ultrasonic receiver was inserted through the right atrium into the right ventricle to approximately the same height as the transmitter. In the right ventricle, which is empty in the isolated perfused Langendorff heart, it was automatically positioned opposite to the transmitter. Additional transducers were placed both at the heart's base and apex for assessment of the ventricular longitudinal diameter.

Several authors used the **isolated perfused mouse heart**.

Bittner et al. (1996) described a work-performing heart preparation for myocardial performance analysis in murine hearts using a modified Langendorff apparatus.

Sumaray and Yellon (1998a, b) constructed a specially designed Langendorff apparatus that allows perfusion of the isolated **mouse** heart. These authors reported that ischemic preconditioning reduces infarct size following global ischemia in the murine myocardium.

Brooks and Apstein (1996) measured left ventricular systolic and diastolic pressures in the isovolumically contracting (balloon in the left ventricle) mouse hearts.

Sutherland et al. (2003) reviewed characteristics and cautions in the use of the isolated perfused heart of mice.

Wang et al. (2001) studied the relationship between ischemic time and ischemia–reperfusion injury in isolated Langendorff-perfused mouse hearts.

Tejero-Taldo et al. (2002) reported that α-adrenergic receptor stimulation produces late preconditioning through inducible nitric oxide synthase in mouse heart.

Ross et al. (2003) found that the α_{1B}-adrenergic receptor decreases the inotropic response in the mouse Langendorff heart model.

Bratkovsky et al. (2004) measured coronary flow reserve in isolated hearts from mice.

Plumier et al. (1995) generated **transgenic mice** expressing the human heart heat shock protein 70. Upon reperfusion of the hearts after 30 min of ischemia in the Langendorff preparation, transgenic hearts versus non-transgenic hearts showed significantly improved recovery of contractile force.

Hannan et al. (2000) compared ENOS knockout and wild-type mouse hearts which were perfused in a Langendorff apparatus with Krebs bicarbonate buffer and subjected to 20 min of global normothermic ischemia followed by 30 min of reperfusion. Myocardial function was measured using a ventricular balloon to determine time to onset of contraction, left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), and heart rate-pressure product (RPP).

Sheikh et al. (2001) generated transgenic mice overexpressing fibroblast growth factor (FGF)-2 protein in the heart. An isolated mouse heart model of ischemia–reperfusion injury was used to assess the potential of endogenous FGF-2 for cardioprotection.

Mouren et al. (2010) showed that endothelium-dependent and endothelium-independent coronary flow responses are increased in Krebs–Henseleit mixed with red blood cell-perfused heart as compared with Krebs–Henseleit-perfused heart. Krebs–Henseleit/red blood cell-perfused hearts are more sensitive for studying the pharmacological response of coronary vasculature to vasoactive drugs. They also showed that the Krebs–Henseleit/red blood cell-perfused heart was able to achieve adequate O_2 supply even when myocardial metabolic demands are markedly increased.

Applications

Positive Inotropic Effects

While negative inotropic substances can be tested in a heart beating with normal force, the evaluation of a positive inotropic compound usually requires that cardiac force is first reduced. Acute experimental heart failure can be induced by an overdose of barbiturates, such as sodium thiopental, or calcium antagonists. This kind of cardiac failure can be reversed by β-sympathomimetic drugs, cardiac glycosides, or increased Ca^{+2} concentration. In this way, the potential β-sympathomimetic activity of a new drug can be measured using isoproterenol as standard. After thiopental-Na treatment, left ventricular pressure (LVP) and dp/dt_{max} decrease considerably, whereas coronary flow is slightly enhanced. β-Sympathomimetic drugs restore LVP and dp/dt_{max} and keep coronary blood flow elevated.

Cardiac glycosides increase LVP and dp/dt_{max} and leave coronary flow unchanged.

Negative Inotropic Effects

The effects of a β-sympathomimetic drug such as isoproterenol at doses of 0.05–0.2 µg increasing contractile force as well as heart frequency are registered. After injection of a β-blocker, the effects of isoproterenol are attenuated. The effects of a potential β-blocking agent can be tested comparing the isoproterenol inhibition versus a standard such as propranolol (0.1 mg).

Coronary Vessel Dilating Effect

The Langendorff heart has been extensively used for assessing the coronary dilating activity of drugs (Broadley 1979). Rothaul and Broadley (1982) demonstrated the release of coronary vasodilator mediators from guinea pig isolated hearts by a technique employing donor and recipient hearts in series.

Calcium Antagonism

In order to demonstrate the effect of calcium antagonists, 1–5 mg $BaCl_2$ is injected which induces a pronounced spasm of the coronary arteries, thereby reducing the coronary flow. Five min later, the test drug is injected. Active compounds have a relaxing effect on coronary arteries indicated by an increase of coronary flow. After this effect has weaned, $BaCl_2$ is injected again and the test drug or a standard drug, e.g., nifedipine, is tested. The increase of coronary flow is expressed as percentage of flow during $BaCl_2$ spasm and compared with the effect of the standard. Using

various doses, dose–response curves can be established.

Effect on Potassium Outflow Induced by Cardiac Glycosides

Lindner and Hajdu (1968) described a method using the Langendorff heart in which contractile force, coronary flow, and the potassium content in the coronary outflow were determined by flame photometry. Increase in potassium outflow correlates well with the positive inotropic effect.

Gradual Determination of Hypoxic Damage

Lindner and Grötsch (1973) measured the enzymes creatine phosphokinase (CPK), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (α-HBDH), and glutamic oxalacetic transaminase (GOT) in the effluent of a guinea pig heart preparation under varying degrees of hypoxia. Potassium content and oxygen tension in the inflowing and outflowing solution were determined. The heart rate, the amplitude of contraction, and the rate of coronary vessel perfusion were recorded additionally.

Metabolic Studies with Nuclear Magnetic Resonance

Using ^{31}P, studies on metabolism of nucleotides and phosphorylated intermediates of carbohydrates in isolated hearts have been performed (Garlick et al. 1977; Jacobus et al. 1977; Hollis et al. 1978; Matthews and Radda 1984).

Arrhythmogenic, Antiarrhythmic, and Antifibrillatory Effects

The Langendorff heart preparation is also used to test the influence of compounds on cardiac rhythm. For recording monophasic action potentials, suction electrodes are applied on the heart. Ventricular fibrillation can be induced by simultaneous injection of digitoxin (12.5–25.0 µg) and aconitine (12.5–25.0 µg) into the perfusion fluid (Lindner 1963). Cardiac glycosides shorten the refractory period, decrease the conduction velocity and increase heterotopic stimulus generation. Aconitine increases markedly heterotopic stimulus generation. Both compounds together induce invariably ventricular fibrillation. Antiarrhythmic compounds can be tested in this way. Fibrillation is inhibited, at least partially, by 20 µg prenylamine, 10–20 µg quinidine, or 20 µg ajmaline.

Takeo et al. (1992) described protective effects of antiarrhythmic agents on oxygen-deficiency-induced contractile dysfunction of isolated perfused hearts. Hypoxia in isolated rabbit hearts was induced by perfusing the heart for 20 min with Krebs–Henseleit buffer saturated with a gas mixture of 95 % N_2 and 5 % CO_2 containing 11 mM mannitol. After hypoxic perfusion, the heart was reoxygenated for 45 min with oxygenated buffer containing glucose.

Dhein et al. (1989) studied the pathway and time course of the epicardial electrical activation process by means of a computer-assisted epicardial potential mapping, using a matrix of 256 unipolar AgCl electrodes (1 mm spatial and 0.25 ms temporal resolution) in isolated rabbit hearts perfused according to the Langendorff technique. From the activation times of the surrounding electrodes, the direction and velocity of activation for each electrode were calculated, thereby allowing construction of an epicardial vector field. The method was used for the assessment of arrhythmogenic and antiarrhythmic drug activity.

Electrical Stimulation and Antifibrillatory Effect

Ventricular fibrillation can be induced in the Langendorff preparation by reducing the glucose content of the perfusion medium to 0.25 g/1,000 ml and the KCl content to 0.12 g/1,000 ml (Burn and Goodford 1957; Burn and Hukovic 1960; Lindner 1963). After a perfusion period of 20 min, 10 µg epinephrine is injected into the perfusion cannula. Immediately afterwards, the heart is stimulated with a current of 40 Hz and 5 mA for 2 min. This procedure is repeated every 10 min. Standard conditions are achieved when the fibrillation continues without further electrical stimulation. Hearts treated in this way serve as controls. Other hearts stimulated in the same way are treated with continuous infusion of the test drug or the standard via the perfusion medium. Differences in the incidence of fibrillations are calculated using the χ^2 test.

Electrophysiological Evaluation of Cardiovascular Agents

Balderston et al. (1991) modified the Langendorff technique in rabbit hearts in order to perform electrophysiologic studies. His bundle electrograms were measured with a plunge electrode and allowed atrioventricular nodal physiology to be evaluated directly. Atrial conduction and refractoriness, atrioventricular node conduction and refractoriness, His–Purkinje conduction, and ventricular conduction and refractoriness could be accurately measured. The effects of verapamil and flecainide were described.

EDRF Release from the Coronary Vascular Bed

Lamontagne et al. (1992) isolated platelets from the blood of healthy human donors and injected platelet boluses into the perfusion line of the Langendorff preparation of a rabbit heart. In the effluent, cyclic GMP was determined as an index for EDRF release.

References and Further Reading

Avkiran M, Curtis MJ (1991) Independent dual perfusion of left and right coronary arteries in isolated rat hearts. Am J Physiol 261(Heart Circ Physiol 30):H2082–H2090

Balderston SM, Johnson KE, Reiter MJ (1991) Electrophysiologic evaluation of cardiovascular agents in the isolated intact rabbit heart. J Pharmacol Methods 25:205–213

Bardenheuer H, Schrader J (1983) Relationship between myocardial oxygen consumption, coronary flow, and adenosine release in an improved isolated working heart preparation of guinea pigs. Circ Res 51:263–271

Barr RL, Lopaschuk GD (1997) Direct measurement of energy metabolism in the isolated rat heart. J Pharmacol Toxicol Methods 38:11–17

Bittner HB, Chen EP, Peterseim DS, Van Trigt P (1996) A work-performing heart preparation for myocardial performance analysis in murine hearts. J Surg Res 64:57–62

Bratkovsky S, Aasum E, Birkeland CH, Riemersma RA, Myrhe ESP, Larsen TS (2004) Measurement of coronary flow reserve in isolated hearts from mice. Acta Physiol Scand 181:167–172

Broadley KJ (1979) The Langendorff heart preparation – reappraisal of its role as a research and teaching model for coronary vasoactive drugs. J Pharmacol Methods 2:143–156

Brooks WW, Apstein CS (1996) Effect of treppe on isovolumic function in the isolated blood-perfused mouse heart. J Mol Cell Cardiol 28:1817–1822

Brown TG, Lands AM (1964) Cardiovascular activity of sympathomimetic amines. In: Laurence DR, Bacharach AL (eds) Evaluation of drug activities: pharmacometrics. Academic Press, London/New York, pp 353–368

Burn HJ, Goodford PJ (1957) Effect of lack of glucose and of lack of oxygen on ventricular fibrillation. J Physiol 137:20P–21P

Burn HJ, Hukovic S (1960) Anoxia and ventricular fibrillation: with a summary of evidence on the cause of fibrillation. Br J Pharmacol 15:67–70

Chevalier B, Mouas C, Mansier P, Aumont MC, Swynghedauw B (1987) Screening of inotropic drugs on isolated rat and guinea pig hearts. J Pharmacol Methods 17:313–326

Dhein S, Müller A, Klaus W (1989) The potential of epicardial activation mapping in isolated hearts for the assessment of arrhythmogenic and antiarrhythmic drug activity. J Pharmacol Methods 22:197–206

Döring HJ (1990) The isolated perfused warm-blooded heart according to LANGENDORFF. Technique – function – application. Physiologie Bohemoslov 39:481–496

Flynn SB, Gristwood RW, Owen DAA (1978) Characterization of an isolated, working heart guinea-pig heart including effects of histamine and noradrenaline. J Pharmacol Methods 1:183–195

Garlick PP, Radda GK, Seeley PJ, Chance B (1977) Phosphorus NMR studies on perfused heart. Biochem Biophys Res Commun 74:1256–1262

Gottlieb R, Magnus R (1904) Digitalis und Herzarbeit. Nach Versuchen an überlebenden Warmblüterherzen. Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 51:30–63

Hannan RL, John MC, Kouretas PC, Hack BD, Matherne GP, Laubach VE (2000) Deletion of endothelial nitric oxidase exacerbates

myocardial stunning in an isolated mouse heart model. J Surg Res 93:127–132

Hendrikx M, Mubagawa K, Verdonk F, Overloop K, Van Hecke P, Vanstapel F, Van Lommel A, Verbeken E, Lauweryns J, Flameng W (1994) Na^{+}-H^{+} exchange inhibitor HOE 694 improves postischemic function and high-energy phosphate resynthesis and reduces Ca^{2+} overload in isolated perfused rabbit heart. Circulation 89:2787–2798

Hollis DP, Nunnally RL, Taylor GJ, Weisfeldt ML, Jacobus WE (1978) Phosphorus NMR studies of heart physiology. J Magn Reson 29:319–330

Hukovic S, Muscholl E (1962) Die Noradrenalin-Abgabe aus dem isolierten Kaninchenherzen bei sympathischer Nervenreizung und ihre pharmakologische Beeinflussung. Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 244:81–96

Igic R (1996) The isolated perfused "working" rat heart: a new method. J Pharmacol Toxicol Methods 35:63–67

Ishiu R, Abe Y, Onishi K, Ueda Y, Sekioka K, Nakano T (1996) Changes in calcium transient and left ventricular function during inotropic stimulation and myocardial ischemia in indo-1-loaded beating guinea pig heart. J Pharmacol Toxicol Methods 35:55–61

Jacobus WE, Taylor GJ, Hollins DP, Nunnally RL (1977) Phosphorus nuclear magnetic resonance of perfused working rat hearts. Nature 265:756–758

Krzeminski T, Kurcok A, Kapustecki J, Kowalinski J, Slowinski Z, Brus R (1991) A new concept of the isolated heart preparation with on-line computerized data evaluation. J Pharmacol Methods 25:95–110

Lamontagne D, König A, Bassenge E, Busse R (1992) Prostacyclin and nitric oxide contribute to the vasodilator action of acetylcholine and bradykinin in the intact rabbit coronary bed. J Cardiovasc Pharmacol 20:652.657

Langendorff O (1895) Untersuchungen am lebenden Säugethierherzen. Pflüger's Arch Ges Physiol 61:291–332

Lindner E (1963) Untersuchungen über die flimmerwidrige Wirkung des *N*-(3′-phenylpropyl-(2′))-1,1-diphenylpropyl-(3)-amins (Segontin). Arch Int Pharmacodyn 146:485–500

Lindner E, Grötsch H (1973) Methode zur graduellen Bestimmung hypoxischer Schädigung am isolierten Meerschweinchenherzen nach Langendorff. Arzneim Forsch/Drug Res 23:926–929

Lindner E, Hajdu P (1968) Die fortlaufende Messung des Kaliumverlustes des isolierten Herzens zur Bestimmung der Wirkungsstärke digitalisartiger Körper. Arch Int Pharmacodyn 175:365–372

Linz W, Martorana PA, Schölkens BA (1990) Local inhibition of bradykinin degradation in ischemic hearts. J Cardiovasc Pharmacol 15(Suppl 6):S99–S109

Linz W, Schölkens BA, Han YF (1986) Beneficial effects of the converting enzyme inhibitor, ramipril, in ischemic rat hearts. J Cardiovasc Pharmacol 8(Suppl 10):S91–S99

Matthews PM, Radda GK (1984) Applications of nuclear magnetic resonance to the study of myocardial metabolism and pharmacology. In: Schwartz A (ed) Methods in pharmacology. Myocardial biology, vol 5. Plenum Press, New York/London, pp 175–228

Michio F, Hideo I, Tetsuya A (1985) *In vitro* assessment of myocardial function using a working rabbit heart. J Pharmacol Methods 14:49–60

Mouren S, Vicaut E, Lamhaut L, Riou B, Ouattara A (2010) Crystalloid versus red blood cell-containing medium in the Langendorff-perfused isolated heart preparation. Eur J Anaesthesiol 27(9):780–787

Neely JR, Liebermeister H, Batterbsy EJ, Morgan HE (1967) Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol 212:804–814

Plumier JCL, Ross BM, Currie RW, Angelidis CA, Kazlaris H, Kollias G, Pagoulatos GN (1995) Transgenic mice expressing the human heart heat shock protein 70 have improved postischemic myocardial recovery. J Clin Invest 95:1854–1860

Ross BD (1972) Chapter 5: Heart and skeletal muscle. In: Perfusion techniques in biochemistry. A

laboratory manual in the use of isolated perfused organs in biochemical experimentation. Clarendon Press, Oxford, pp 258–320

Ross SA, Rorabaugh BR, Chalothorn D, Yun J, Gonzalez-Cabrera PJ, McCune DF, Piasik MT, Perez DM (2003) The α_{1B}-adrenergic receptor decreases the inotropic response in the mouse Langendorff heart model. Cardiovasc Res 60:598–607

Rothaul AL, Broadley KJ (1982) Measurements of oxygen tension in perfusates from guinea pig isolated hearts and the demonstration of coronary vasodilator material. J Pharmacol Methods 7:91–103

Sakai K, Akima M, Tsuyama K (1983) Evaluation of the isolated perfused heart of mice, with special reference to vasoconstriction caused by intracoronary acetylcholine. J Pharmacol Methods 10:263–270

Sheikh F, Sontag DP, Fandrich RR, Kardami E, Cattini PA (2001) Overexpression of FGF-2 increases cardiac myocyte viability after injury in isolated mouse hearts. Am J Physiol Heart Circ Physiol 280:H1030–H1050

Sumaray MS, Yellon DM (1998a) Characterization and validation of a murine model of global ischaemia-reperfusion injury. Mol Cell Biochem 186:61–68

Sumaray MS, Yellon DM (1998b) Ischemic preconditioning reduces infarct size following global ischemia in the murine myocardium. Basic Res Cardiol 93:384–390

Sutherland FJ, Shattock MJ, Baker KE, Hearse DJ (2003) Mouse isolated perfused heart: characteristics and cautions. Clin Exp Pharmacol Physiol 30:867–898

Takeo S, Tanonaka K, Liu JX, Ohtsuka Y (1992) Protective effects of antiarrhythmic agents on oxygen-deficiency-induced contractile dysfunction of isolated perfused hearts. In: Yasuda H, Kawaguchi H (eds) New aspects in the treatment of failing heart. Springer, Tokyo/Berlin/Heidelberg, pp 13–219

Tejero-Taldo MI, Gursoy E, Zhao TC, Kukreja RC (2002) α-Adrenergic receptor stimulation produces late preconditioning through inducible nitric oxide synthase in mouse heart. J Mol Cell Cardiol 34:185–195

Wang QD, Swärdh A, Sjöquist PO (2001) Relationship between ischemic time and ischemia/reperfusion injury in isolated Langendorff-perfused mouse hearts. Act Physiol Scand 171:123–128

Xiang JZ, Linz W, Becker H, Ganten D, Lang RE, Schölkens B, Unger T (1985) Effects of converting enzyme inhibitors: ramipril and enalapril on peptide action and neurotransmission in the isolated heart. Eur J Pharmacol 113:215–223

Zander B, Euler H (1976) Concentration measurements of physically dissolved oxygen by the classical van Slyke principle. In: Deng H, Balslen J, Brook R (eds) Measurement of oxygen. Elsevier Scientific Publ. Co, Amsterdam, pp 271–276

Zegner M, Podesser B, Koci G, Weisser J, Hallström S, Schima H, Wollenek GH (1996) Evaluation of the influences of ramiprilat on the reperfusion – Studied on the isolated working heart model. Acta Chir Austriatica 28:343–346

Coronary Artery Ligation in Isolated Working Rat Heart

Purpose and Rationale

In working heart preparations of rats, ischemia can be induced by clamping the left coronary artery close to its origin. After removal of the clip, changes in the reperfusion period can be observed. Prevention of these symptoms can be an indicator of the efficacy of coronary drugs.

Procedure

The preparation used is a modification of an isolated working heart preparation originally used for guinea pig hearts (Bardenheuer and Schrader 1983). Wistar rats of either sex weighing 280–300 g are sacrificed by decapitation. The hearts are removed and dissected free from the epicard and surrounding connective tissue. A cannula is introduced into the aorta from where the coronary vessels are perfused with the non-recirculated perfusion medium according to the Langendorff technique. In the left ventricle, a

balloon closely fitting the ventricular cavity is placed and connected to an artificial systemic circulation. The fluid in the balloon is pumped through a cannula into the closed extracorporeal circulation being forced into one direction by two recoil valves. The balloon is made of silicone material using a Teflon form (Linz et al. 1986a). The dimensions of the form are derived from casts of the left ventricle of K^+-arrested heart by injection of dental cement (Palavit 55, Kulzer and Co, GmbH, Germany). During each heart beat, the fluid volume pressed from the balloon, corresponding to the stroke volume of the heart, can be recorded by means of a flow meter probe and an integrator connected in series. Preload and afterload can be adjusted independently from each other. The perfusate flow (retrograde into the aorta and through the coronary arteries) is recorded separately.

The following parameters were measured in isolated rat hearts (Linz et al. 1986b):

LVP (left ventricular pressure) with Statham pressure transducer P 23 DB, which on differentiation yielded LV dp/dt_{max} and HR (heart rate). Cardiac output and coronary flow (CF) are determined by electromagnetic flow probes in the outflow system and in the aortic cannula, respectively. Coronary venous pO_2 is measured with a catheter placed in the pulmonary artery by a type E 5046 electrode connected to a PMH 73 pH/blood gas monitor (Radiometer). An epicardial electrocardiogram recording is obtained via two silver electrodes attached to the heart. All parameters are recorded on a Brush 2600 recorder.

Myocardial oxygen consumption (MWO_2) [ml/min/g wet weight] is calculated according to the equation

$$MVO_2 = CF \times (P_a - P_V) \times (c/760) \times 100$$

where CF is the coronary flow [ml/min/g], Pa is the oxygen partial pressure of arterial perfusate (650 mmHg), P_v is the oxygen partial pressure of the venous effluent perfusate [mmHg], and c is the 0.0227 ml O_2/ml perfusate representing the Bunsen solubility coefficient of oxygen dissolved in perfusate at 37 °C (Zander and Euler 1976).

Coronary Artery Ligation

For coronary artery occlusion experiment (Scholz et al. 1992, 1993), the isolated working hearts are perfused for a period of 20 min (pre-ischemic period) with modified Krebs–Henseleit buffer at a constant pressure of 65 mmHg. Thereafter, acute myocardial ischemia is produced by clamping the left coronary artery close to its origin for 15 min (ischemic period). The clip is then reopened, and changes during reperfusion are monitored for 30 min (reperfusion period). After coronary artery ligation and reperfusion, the hearts develop ventricular fibrillation.

From the coronary effluent, samples are taken for lactate, lactate dehydrogenase (LDH), and creatine kinase (CK) determinations. After the experiment, glycogen, lactate, ATP, and creatine phosphate in myocardial tissue are measured.

The test drugs are given into the perfusion medium either before occlusion or 5 min before reperfusion. For ex vivo studies, the rats are treated orally with the test drug 1 h before sacrifice and preparation of the isolated working heart.

Evaluation

The incidence and duration of ventricular fibrillation after treatment with coronary drugs is compared with controls. Left ventricular pressure, LV dP/dt max, and coronary flow are reduced after coronary constriction by angiotensin II, whereas enzyme activities in the effluent are increased and the myocardial content of glycogen, ATP, and creatine phosphate are decreased. Cardiac protective drugs have the opposite effects. The values of each parameter are statistically compared with controls.

Modifications of the Method

Vogel and Lucchesi (1980) described an isolated, blood-perfused, **feline** heart preparation for evaluating pharmacological interventions during myocardial ischemia. Ventricular function was measured with a fluid-filled latex balloon within the left ventricle.

Vleeming et al. (1989) ligated the left coronary artery in rats after thoracotomy in ether anesthesia. Forty-eight hours after the operation, the hearts were prepared for retrograde constant pressure

perfusion, according to the Langendorff technique.

Igic (1996) presented a new method for the isolated working rat heart. A special double cannula was designed consisting of an outer cannula that is inserted in the aorta and an inner cannula that is advanced into the left ventricle. The perfusion fluid flows through the inner cannula into the left ventricle and is ejected from there into the aorta. If the outer cannula system is closed, the fluid perfuses the coronary vessels and drips off outside the heart. When the outer cannula is open and certain pressure resistance is applied, a fraction of the ejected fluid perfuses the coronary vessels and the rest is expelled. Because the inner cannula can easily be retracted into the outer cannula, which is placed in the aorta, this preparation provides an opportunity to use the same heart as a "working" or "nonworking" model for investigating functions of the heart.

Pepe and McLennan (1993) described a maintained afterload model of ischemia in erythrocyte-perfused isolated working hearts of rats.

Further characterization of the pathophysiological reactions or the isolated working heart was performed by Linz et al. (1999). The external heart power (EHP) [mJ/min/g] was calculated using the formula

$$\begin{aligned} EHP_{\mathrm{LV}} &= \text{pressure} - \text{volume} + \text{acceleration work} \\ &= [SV(MAP - LAP)] \\ &\quad + [1/2SV \times d \times (SV/\pi r^2 e^2)]HRg^{-1}_{\mathrm{LVwwt}} \end{aligned}$$

where *SV* indicates stroke volume; *MAP*, mean aortic pressure; *LAP*, mean left arterial pressure; *d*, specific weight perfusate (1.004 g/cm^3); *r*, inner radius of aortic cannula; *e*, ejection time; *HR*, heart rate; LV, left ventricle; and LVwwt, left ventricular wet weight.

The function of the left ventricle was altered by changing the aortic pressure (afterload) at constant left atrial filling load (preload). By adjusting the Starling resistance, the aortic outflow could be switched during 1 min from the fixed baseline afterload to a preset higher afterload producing step-wise rises in mean arterial pressure.

Lee et al. (1988) studied the effects of acute global ischemia on cytosolic calcium transients in perfused isolated **rabbit** hearts with the fluorescent calcium indicator indo 1. Indo 1-loaded hearts were illuminated at 360 nm, and fluorescence was recorded simultaneously at 400 and 550 nm from the epicardial surface of the left ventricle. The F_{400}/F_{550} ratio was calculated by an analog circuit, which allowed cancelation of optical motion artifact. The resulting calcium transients were registered simultaneously with the ventricular pressure and demonstrated a rapid upstroke and slow decay similar to those recorded in isolated ventricular myocytes. Global ischemia rapidly suppressed contraction, but it produced a concurrent increase in the systolic and diastolic levels of calcium transients, together with an increase in the duration of the peak.

References and Further Reading

Grupp IL, Grupp G (1984) Isolated heart preparations perfused or superfused with balanced salt solutions. In: Schwartz A (ed) Methods in pharmacology. Myocardial biology, vol 5. Plenum Press, New York/London, pp 111–128

Igic R (1996) The isolated perfused "working" rat heart: a new method. J Pharmacol Toxicol Methods 35:63–67

Kannengieser GJ, Lubbe WF, Opie LH (1975) Experimental myocardial infarction with left ventricular failure in the isolated perfused rat heart. Effects of isoproterenol and pacing. J Mol Cell Cardiol 7:135–151

Lee HC, Mohabir R, Smith N, Franz MR, Clusin WT (1988) Effect of ischemia on calcium-dependent fluorescence transients in rabbit hearts containing indo 1. Correlation with monophasic action potentials and contraction. Circulation. 78(4):1047–59.

Linz W, Schölkens BA, Han YF (1986a) Beneficial effects of the converting enzyme inhibitor, ramipril, in ischemic rat hearts. J Cardiovasc Pharmacol 8(Suppl 10):S91–S99

Linz W, Schölkens BA, Manwen J, Wilhelm M, Ganten D (1986b) The heart as a target for converting enzyme inhibitors: studies in

ischaemic isolated working hearts. J Hypertens 4(Suppl 6):S477–S479
Linz W, Schölkens BA, Kaiser J, Just M, Bei-Yin Q, Albus U, Petry P (1989) Cardiac arrhythmias are ameliorated by local inhibition of angiotensin formation and bradykinin degradation with the converting-enzyme inhibitor ramipril. Cardiovasc Drugs Ther 3:873–882
Linz W, Wohlfart P, Schoelkens BA, Becker RHA, Malinski T, Wiemer G (1999) Late treatment with ramipril increases survival in old spontaneously hypertensive rats. Hypertension 34:291–295
Martorana PA, Linz W, Göbel H, Petry P, Schölkens BA (1987) Effects of nicainoprol on reperfusion arrhythmia in the isolated working rat heart and on ischemia and reperfusion arrhythmia and myocardial infarct size in the anesthetized rat. Eur J Pharmacol 143:391–401
Pepe S, McLennan PL (1993) A maintained afterload model of ischemia in erythrocyte-perfused isolated working hearts. J Pharmacol Toxicol Methods 29:203–210
Rajagopalan R, Ghate AV, Subbarayan P, Linz W, Schoelkens BA (1993) Cardiotonic activity of the water soluble forskolin derivative 8,13-epoxy-6β-(piperidinoacetoxy)-1α,7β,9α-trihydro-xy-labd-14-en-11-one. Arzneim Forsch/Drug Res 43:313–319
Schölkens BA, Linz W, Lindpaintner K, Ganten D (1987) Angiotensin deteriorates but bradykinin improves cardiac function following ischaemia in isolated rat hearts. J Hypertens 5(Suppl 5): S7–S9
Scholz W, Albus U, Linz W, Martorana P, Lang HJ, Schölkens BA (1992) Effects of Na^+/H^+ exchange inhibitors in cardiac ischaemia. J Mol Cell Cardiol 24:731–740
Scholz W, Albus U, Lang HJ, Linz W, Martorana PA, Englert HC, Schölkens BA (1993) Hoe 694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischemia. Br J Pharmacol 109:562–568
van Gilst WH, de Graeff PA, Wesseling H, de Langen CDJ (1986) Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors: a comparison of captopril, enalapril, and HOE 498. J Cardiovasc Pharmacol 8:722–728
Vleeming W, van der Wouw PA, van Rooij HH, Wemer J, Porsius AJ (1989) *In vitro* method for measurement of cardiac performance and responses to inotropic drugs after experimentally induced myocardial infarction in the rat. J Pharmacol Methods 21:95–102
Vogel WM, Lucchesi BR (1980) An isolated, blood perfused, feline heart preparation for evaluating pharmacological interventions during myocardial ischemia. J Pharmacol Methods 4:291–303

Isolated Working Heart Model in Infarcted Rat Heart

Purpose and Rationale

The model of chronic heart failure in spontaneously hypertensive rats described by Itter et al. (2004a ▶ Methods to Induce Cardiac Hypertrophy and Insufficiency) has been used by the same group to study the isolated working heart in rats after chronic infarction (Itter et al. 2004b).

Procedure

Animals and Methods

WKY/NHsd and SHR/NHsd rats at an age of 4 months were randomized into two groups – sham and myocardial infarction (MI). The sham procedure consisted of opening the pericardium and placing a superficial suture in the epicardium of the left ventricle (LV). Chronic heart failure (CHF) was induced by permanent (8 weeks) occlusion of the left coronary artery 2 mm distal to the origin from the aorta resulting in a large infarction of the free left ventricular wall.

Eight weeks after surgery, parameters indicating CHF were measured. Cardiac hypertrophy, function, and geometric properties were determined by the "working heart" mode and in vivo determinations by MRI and heart weight.

Surgery

The rats were anesthetized with a mixture of ketamine/xylazine (35/2 mg/kg i.p.). The left ventrolateral thorax was shaved and prepared to create a

disinfected surgical access area. When stable anesthesia was achieved, the animals were placed on a small animal operation table, intubated, and ventilated with room air using a small animal ventilator (KTR-4, Hugo Sachs Elektronik, March-Hugstetten, Germany). The level of anesthesia was deemed as adequate following loss of the pedal withdrawal reflex and absence of the palpebral reflex. Reflexes were evaluated before surgery. The operation took 5 min. The tidal volume was adjusted at 3–5 ml and the ventilation rate was 40 breaths/min. Left thoracotomy was performed via the third intercostal space. The heart was exposed and the pericardium opened. The left main coronary artery was ligated with Perma-Hand silk 4–0 USP (Ethicon, Nordersredt, Germany) near its origin at the aorta (2 mm distal to the edge of the left atrium). Ligation resulted in infarction of the free left ventricular wall. Ligation was deemed successful when the anterior wall of the left ventricle turned pale. At this point, the lungs were hyperinflated by increasing the positive end-expiratory pressure, and the chest was closed. The rats were placed on a heating pad and covered with a layer of unbleached tissue paper. The rats were extubated following return of reflexes. They were continuously monitored until they started moving in their cages. To avoid ventricular arrhythmias, lidocaine (2 mg/kg i.m.) was given before surgery. To prevent acute lung edema, the rats received furosemide (Lasix, 2 mg/kg bodyweight twice daily for 3 days) via the drinking water. To avoid pain and distress, the rats received metamizole treatment (Novalgin, 0.1 mg/kg body weight i.m.) once, directly after the recovery period.

Before killing the animals 8 weeks after MI, noninvasive sequential nuclear magnetic resonance (NMR) measurements of heart geometric properties were done. Thereafter, the animals were anesthetized with pentobarbital (180 mg/kg i.p. pentobarbital) and subsequently heparinized (heparin natrium 500 I.U./100 g body weight i.p.). Once stable anesthesia was achieved (stage III 3, reflexes absent), the animals were connected to an artificial respirator via a PE (polyethylene) tube inserted into the trachea and ventilated with room air. A transverse laparotomy and a right anterolateral thoracotomy were performed, and the heart was rapidly removed for the evaluation of its function in the working heart mode. The heart was immersed in physiological buffer chilled to 4 °C. The aorta was dissected free and mounted onto a cannula (internal diameter: 1.4 mm) attached to a perfusion apparatus. The hearts were perfused according to the method of Langendorff with an oxygenated (95 % O_2/5 % CO_2) non-circulating Krebs–Henseleit solution of the following compositions (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$, 1.64; $NaHCO_3$, 24.88; KH_2PO_4, 1.18; glucose, 5.55; and Na pyruvate, 2.0 at a perfusion pressure of 60 mmHg. Any connective tissue in the thymus or lung was carefully removed. A catheter placed into the pulmonary artery drained the coronary effluent perfusate that was collected for the determination of coronary flow and venous pO_2 measurements. The left atrium was cannulated via an incision of the left auricle. All pulmonary veins were ligated close to the surface of the atria.

When a tight seal with no leaks had been established and after a 15 min equilibration period, the hearts were switched into the working mode, using a filling pressure (preload) of 12 mmHg in WKY/NHsd and 18 mmHg in SH rats. The afterload pressure was 60 mmHg in WKY/NHsd and 80 mmHg in SH rats. After validation of the basis parameters, the afterload pressure was enhanced in a cumulative manner from an additional 20–140 mmHg. Thereafter, the isovolumetric maxima were determined by enhancing the preload pressure in steps of 5–30 mmHg.

Flow and pressure signals for computation were obtained from the PLUGSYS-measuring system (Hugo Sachs Elektronik, March-Hugstetten, Germany). Computation of data was performed with a sampling rate of 500 Hz, averaged every 2 s, using the software Aquire Plus V1.21f (PO-NE-MAH, Hugo Sachs Elektronik, March-Hugstetten, Germany).

Determination of Infarct Size

After the evaluation of the external heart work, the total heart weight and the left and right ventricular weights were determined. The left ventricle was

then sectioned transversely into four slices from the apex to the base. Eight pictures were taken of each rat heart, two from each slice. Total infarct size was determined by planimetry of the projected and magnified slices. The areas of infarcted tissue as well as the intact myocardium of each slice were added together and averaged. The infarcted fraction of the left ventricle was calculated from these measurements and expressed as a percentage of the left ventricular mass. The left ventricular perimeter, diameter, infarct scar length, as well as wall thickness and infarct wall thinning were determined as well.

Evaluation

The data are given as mean ± SEM. Statistics were performed using the SAS system statistics package (SAS Institute, Cary, N.C., USA) with a sequential rejection *t*-test according to Holm (1979).

References and Further Reading

Holm S (1979) A simple sequential rejective multiple test procedure. Scand J Stat 6:65–70

Itter G, Jung W, Juretschke P, Schölkens BA, Linz W (2004a) A model of chronic heart failure in spontaneous hypertensive rats (SHR). Lab Anim 38:138–148

Itter G, Jung W, Schölkens BA, Linz W (2004b) The isolated working heart model in infarcted rat hearts. Lab Anim 39:178–193

Relaxation of Bovine Coronary Artery

Purpose and Rationale

Eicosanoids can regulate the tonus of coronary arteries. Prostacyclin induces relaxation, whereas thromboxane A_2 causes contraction. Spiral strips from bovine coronary artery can be used for assaying relaxation activity of test compounds (Dusting et al. 1977).

Procedure

Freshly slaughtered beef hearts are immersed in cold oxygenated Krebs solution and immediately transported in a thermos flask to the laboratory. The left descending coronary artery and several of its primary branches are cut into spiral strips (about 20 mm long and 2–3 mm wide). The specimens can be stored up to 48 h at 4 °C. The artery strips are suspended in a 4 ml organ bath under an initial tension of 2 g and immersed in a Krebs' bicarbonate solution at 37 °C being gassed with oxygen containing 5 % CO_2 throughout the experiment. The Krebs solution contains a mixture of antagonists to inhibit any actions from endogenous acetylcholine, 5-hydroxytryptamine, histamine, or catecholamines (hyoscine hydrobromide 10^{-7} g/ml, methysergide maleate 2×10^{-7} g/ml, mepyramine maleate 10^{-7} g/ml, propranolol hydrochloride 2×10^{-6} g/ml). The strips are superfused with a solution of the test compounds in concentrations of 0.01, 0.1, and 1.0 µg/ml at a rate of 10–20 ml/min with oxygenated Krebs solution containing the mixture of antagonists. Isometric contractions are recorded with Grass force-displacement transducers (type FT 03C) on a Grass polygraph. The strips are superfused with Krebs' solution 3 h prior to the experiment. Standard compounds are 100 ng/ml PGE_2 inducing contraction and 100 ng/ml PGI_2 inducing pronounced relaxation.

Evaluation

The relaxation induced by the test compound is expressed as percentage of maximal response to 100 ng/ml PGI_2.

Modifications of the Method

Campell and Paul (1993) measured the effects of diltiazem on isometric force generation, $[Ca^{2+}]_i$, and energy metabolism in the isolated **porcine** coronary artery.

Li et al. (1997) determined the ability of analogs of human α-calcitonin gene-related peptide to relax isolated porcine coronary arteries precontracted with 20 mM KCl.

References and Further Reading

Dusting GJ, Moncada S, Vane JR (1977) Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins 13:3–15

Gilmore N, Vane JR, Wyllie JH (1968) Prostaglandins released by the spleen. Nature 218:1135–1140

Li J, Matsuura JE, Waugh DJJ, Adrian TE, Abel PW, Manning MC, Smith DD (1997) Structure–activity studies on position 14 of human α-calcitonin gene-related peptide. J Med Chem 40:3071–3076

In Vivo Methods

Isoproterenol-Induced Myocardial Necrosis in Rats

Purpose and Rationale

Cardiac necrosis can be produced by injection of natural and synthetic sympathomimetics in high doses. Infarct-like myocardial lesions in the rat by isoproterenol have been described by Rona et al. (1959). These lesions can be totally or partially prevented by several drugs such as sympatholytics or calcium antagonists.

Procedure

Groups of 10 male Wistar rats weighing 150–200 g are pretreated with the test drug or the standard either s.c. or orally for 1 week. Then, they receive 5.25 and 8.5 mg/kg isoproterenol s.c. on two consecutive days. Symptoms and mortality in each group are recorded and compared with those of rats given isoproterenol alone. Forty-eight hours after the first isoproterenol administration, the rats are sacrificed and autopsied. The hearts are removed and weighed, and frontal sections are embedded for histological examination.

Evaluation

Microscopic examination allows the following grading:

Grade 0: no change
Grade 1: focal interstitial response
Grade 2: focal lesions in many sections, consisting of mottled staining and fragmentation of muscle fibers
Grade 3: confluent retrogressive lesions with hyaline necrosis and fragmentation of muscle fibers and sequestrating mucoid edema
Grade 4: massive infarct with occasionally acute aneurysm and mural thrombi

For each group, the main grade is calculated with the standard deviation to reveal significant differences.

Critical Assessment of the Method

The test has been used by many authors for evaluation of coronary active drugs, such as calcium antagonists and other cardioprotective drugs like nitroglycerin and molsidomine (Vértesi et al. 1991; Classen et al. 1993).

Modifications of the Method

Yang et al. (1996) reported a protective effect of human adrenomedullin$^{13–52}$, a C-terminal fragment of adrenomedullin$^{10–52}$ on the myocardial injury produced by subcutaneous injection of isoproterenol into rats.

References and Further Reading

Bhargava AS, Preus M, Khater AR, Günzel P (1990) Effect of iloprost on serum creatine kinase and lactate dehydrogenase isoenzymes after isoprenaline-induced cardiac damage in rats. Arzneim Forsch/Drug Res 40:248–252

Brodowicz GR, Lamb DR (1991) Exercise training, indomethacin, and isoproterenol-induced myocardial necrosis in rats. Basic Res Cardiol 86:40–48

Campell JD, Paul RJ (1993) Effects of diltiazem on force, $[Ca^{2+}]_i$, and energy metabolism in porcine coronary artery. J Cardiovasc Pharmacol 22:408–415

Ciplea AG, Kretschmar R, Heimann W, Kirchengast M, Safer A (1988) Protective effect of the new calcium antagonist Anipamil against isoprenaline-induced cardionecrosis in rats. Arzneim-Forsch/Drug Res 38:215–221

Classen L, Michalsky G, Kammermeier H (1993) Catecholamine-induced cardiac necroses: protective effect of leucocytopenia, influence of an S2 antagonist, thromboxane synthetase

inhibitor and prostacyclin analogue. Basic Res Cardiol 88:52–59
Ferrans VJ, Hibbs RG, Black WC, Weilbaecher DG (1964) Isoproterenol-induced myocardial necrosis. A histochemical and electron microscopic study. Am Heart J 68:71–90
Genovese A, Chiariello M, de Alfieri W, Latte S, Ferro G, Condorelli M (1982) Quantitative assessment of infarct size in isoproterenol-infarcted rats. Jpn Heart J 23:997–1006
Handforth CP (1962) Isoproterenol-induced myocardial infarction in animals. Arch Pathol 73:161–165
Joseph X, Bloom S, Pledger G, Balazs T (1983) Determinants of resistance to the cardiotoxicity of isoproterenol in rats. Toxicol Appl Pharmacol 69:199–205
Knufman NMJ, van der Laarse A, Vliegen HW, Brinkman CJJ (1987) Quantification of myocardial necrosis and cardiac hypertrophy in isoproterenol-treated rats. Res Commun Chem Pathol Pharmacol 57:15–32
Meijer AEFH, Hettwer H, Ciplea AG (1988) An enzyme histochemical study of isoproterenol-induced myocardial necroses in rats. Histochem J 20:697–707
Preus M, Bhargava AS, Khater AER, Günzel P (1988) Diagnostic value of serum creatine kinase and lactate dehydrogenase isoenzyme determinations for monitoring early cardiac damage in rats. Toxicol Lett 42:225–253
Rona G (1967) Experimental drug-induced myocardial infarction for animal pharmacologic screening. In: Siegeler PE, Moyer JH (eds) Animal and clinical pharmacologic techniques in drug evaluation, vol II. Year Book Medical, Chicago, pp 464–470
Rona G, Chappel CI, Balazs T, Gaudry R (1959) An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Arch Pathol 76:443–455
Rona G, Chappel CI, Kahn DS (1963) The significance of factors modifying the development of isoproterenol-induced myocardial necrosis. Am Heart J 66:389–395
Vértesi C, Knopf E, Gaál KPS (1991) Comparison of the cardioprotective effects of nitroglycerin, molsidomine, and SIN-1 in rats. J Cardiovasc Pharmacol 17(Suppl 3): S141–S144
Wexler BC (1985) Prolonged protective effects following propranolol withdrawal against isoproterenol-induced myocardial infarction in normotensive and hypertensive rats. Br J Exp Pathol 66:143–154
Yang J, Zhao D, Chang YZ, Tin Q, Zhao YT, Shi XY, Zhang ZK, Tang CS (1996) Protective effect of adrenomedullin$^{10-52}$ on isoproterenol-induced myocardial injury in rats. Chin Pharmacol Bull 12:530–533

Myocardial Infarction After Coronary Ligation in Rodents

Purpose and Rationale

Ligation of the left coronary artery in rats as described by Selye et al. (1960) induces an acute reduction in pump function and a dilatation of left ventricular chamber. The method has been used to evaluate beneficial effects of drugs after acute (Chiariello et al. 1980; Flaim and Zelis 1981; Bernauer 1985) or chronic (Innes and Weisman 1981; Pfeffer et al. 1985; Linz et al. 1996) treatment.

Procedure

Male Sprague–Dawley rats weighing 200–300 g are anesthetized with diethyl ether. The chest is opened by a left thoracotomy, and a thread is inserted near the middle of the lateral margin of the cutaneous wound and carried through a tunnel of the left pectoral muscle around the cranial half of the incision. The heart is gently exteriorized by pressure on the abdomen. A ligature is placed around the left coronary artery, near its origin, and is tightened. Within seconds, the heart is repositioned in the thoracic cavity, and the ends of the musculocutaneous thread are tightened to close the chest wall and enable the animal to breathe spontaneously.

The speed of the procedure renders mechanical respiration unnecessary.

To evaluate drug effects, the rats are treated 5 min after and 24 h after occlusion by subcutaneous injection (standard 5 mg/kg propranolol).

Two days after surgery, the rats are anesthetized with 60 mg/kg i.p. pentobarbital, and the right carotid artery is cannulated with a polyethylene catheter connected to a pressure transducer. The fluid-filled catheter is then advanced into the left ventricle through the aortic valve for measurement of left ventricular systolic and end-diastolic pressure.

After hemodynamic measurements, the heart is arrested by injecting 2 ml of 2.5 M potassium chloride. The chest is opened, and the hearts are isolated and rinsed with 300 mM KCl to maintain a complete diastole. A double-lumen catheter is advanced into the left ventricle through the ascending aorta, the right and left atria are tied off with a ligature, and the right ventricle is opened. The left ventricular chamber is filled with a cryostatic freeze medium through the smaller of the two catheter lumens and connected to a hydrostatic pressure reservoir maintained at a level corresponding to the end-diastolic pressure measured in vivo. The outlet (larger lumen) is then raised to the same level as the inlet to allow fluid in the two lumens to equilibrate. The heart is rapidly frozen with hexane and dry ice.

The hearts are serially cut with a cryostat into 40 μm thick transverse sections perpendicularly to the longitudinal axis from apex to base. At a fixed distance, eight sections are obtained from each heart and collected on gelatin-coated glass slides. Sections are air-dried and incubated at 25 °C for 30 min with 490 μM nitroblue tetrazolium and 50 mM succinic acid in 0.2 M phosphate buffer (pH 7.6), rinsed in cold distilled water, dehydrated in 95 % ethyl alcohol, cleared in xylene, and mounted with a synthetic resin medium. Viable tissue appears dark blue, contrasting with the unstained necrotic tissue.

Evaluation

The infarct size can be determined by planimetry and expressed as percentage of left ventricular area, and thickness can be expressed as percentage of noninfarcted ventricular wall thickness (MacLean et al. 1978; Chiariello et al. 1980; Roberts et al. 1983). An automatic method for morphometric analysis with image acquisition and computer processing was described by Porzio et al. (1995).

Critical Assessment of the Method

Myocardial infarction following coronary artery ligation in Sprague–Dawley rats is a widely used rat model of heart failure. If the left coronary artery is not completely ligated, heart failure may occur as a consequence of chronic myocardial ischemia (Kajstura et al. 1994).

Modifications of the Method

Johns and Olson (1954) described the coronary artery patterns for mouse, rat, hamster, and guinea pig.

Kaufman et al. (1959) and Fishbein et al. (1978, 1980) used various histochemical methods for the identification and quantification of border zones during the evolution of myocardial infarction.

Sakai et al. (1981) described an **experimental model of angina pectoris in the intact anesthetized rat**. In anesthetized rats, the tip of a special carotid cannula was placed closely to the right and left coronary ostium. Single intra-aortic injections of methacholine or acetylcholine (in the presence of physostigmine) developed a reproducible elevation of the ST segment and the T wave of the electrocardiogram. Coronary drugs were tested to prevent these changes.

Ytrehus et al. (1994) analyzed the effects of anesthesia, perfusate, risk zone, and method of infarct sizing in rat and rabbit heart infarction.

Leprán et al. (1981) placed a loose ligature of atraumatic silk around the left anterior descending coronary artery under ether anesthesia in **rats**. Ten days later, acute myocardial infarction was produced by tightening the ligature.

Kouchi et al. (2000) found an increase in $G_{i\alpha}$ protein accompanying progression of postinfarction remodeling in hypertensive cardiomyopathy in **rats**. G protein α subunits were studied with immunoblotting techniques (Böhm et al. 1990). The polyclonal antiserum MB1 was raised in rabbits against the carboxyl-terminal decapeptide of retinal transduction (KENLKDCGLF) coupled to keyhole limpet hemocyanin. The MB1 recognized $G_{i\alpha 1}$ and $G_{i\alpha 2}$ but not $G_{0\alpha}$ and $G_{i\alpha 3}$ (Böhm et al. 1994). The membrane fractions were electrophoresed in SDS-polyacrylamide gels and were transferred to nitrocellulose filters. The filters were incubated with

the first antibodies for $G_{i\alpha}$ (MB1) or $G_{s\alpha}$ (RM/1) and then with the second antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG, Amersham). Immunoreactive signals were detected by means of the ECL kit (Amersham).

Liu et al. (1997) found that ligation of the left descending coronary artery in Lewis inbred **rats** produces an uniformly large infarct with low mortality. The model may be superior to the usual model in Sprague–Dawley rats with a marked variability in infarct size and cardiac dysfunction.

Coronary artery ligation induces left ventricular remodeling with cardiomyocyte apoptosis and myocardial fibrosis indicated by morphological studies and by collagen accumulation, which can be prevented by drug treatment (Yang et al. 1992; Belichard et al. 1994; Nguyen et al. 1998; Sia et al. 2002; Bäcklund et al. 2004).

The naturally occurring peptide ***N*-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP)** is an inhibitor of pluripotent hematopoietic stem cell proliferation and is normally present in human plasma and circulating mononuclear cells. It is cleaved to an inactive form by the NH_2-terminal catalytic domain of ACE (Azizi et al. 1996). Acute angiotensin-converting enzyme inhibition increases the plasma level of *N*-acetyl-seryl-aspartyl-lysyl-proline (Azizi et al. 1996). By morphological studies and collagen determinations, Rasoul et al. (2004) found an antifibrotic effect of Ac-SDKP and angiotensin-converting enzyme inhibition in hypertension in rats. Similarly, Yang et al. (2004) found that Ac-SDKP reverses inflammation and fibrosis in rats with heart failure after myocardial infarction.

Moreover, Azizi et al. (1997) and Le Meur et al. (1998) discussed whether the plasma Ac-SDKP level is a reliable marker of chronic angiotensin-converting enzyme inhibition in hypertensive patients. An Ac-SDKP EIA Kit is available from Cayman, Ann Arbor, Mich., USA.

Chen et al. (2004) found inhibition and reversal of myocardial infarction-induced hypertrophy and heart failure by NHE-1 inhibition.

Johns and Olson (1954) described a method of experimental **myocardial infarction by coronary occlusion in small animals**, such as **mouse**, **hamster**, **rat**, and **guinea pig**.

Scholz et al. (1995) described a dose-dependent reduction of myocardial infarct size in **rabbits** by a selective sodium–hydrogen exchange subtype 1 inhibitor.

Gomoll and Lekich (1990) tested the **ferret** for a myocardial ischemia/salvage model. Varying combinations of duration of left anterior descending coronary occlusion and reperfusion were evaluated.

Kim et al. (2014) compare the image properties of PET scans obtained using a recently developed 18F-labeled phosphonium cations (Kim et al. 2012) with those images obtained using the gold standard PET myocardial tracer [13N]NH3 in rat myocardial infarction models. [18F]-Labeled (6-Fluorohexyl)triphenylphosphonium cation showed to be a useful replacement for in cardiac PET/CT applications.

Coronary Artery Ligation in Mice

Michael et al. (1995, 1999) and Gould et al. (2001) described the surgical procedure to induce myocardial ischemia in **mice** by ligation of the left anterior descending branch of the left coronary artery.

Infarct and Reperfusion Model

Male C57BL/6 mice 12–16 weeks of age (22.5–30.5 g body weight) were used. Anesthesia was produced by an intraperitoneal injection of pentobarbital sodium (4 mg/ml; 10 μl/g body weight). Mice were placed in a supine position with paws taped to the operating table. With direct visualization of the trachea, an endotracheal tube was inserted and connected to a Harvard rodent volume-cycled ventilator cycling at 100/min with volume sufficient to adequately expand the lungs but not overexpand. The inflow valve was supplied with 100 % oxygen.

For studies of the myocardial response to permanent occlusion, ligation of the anterior descending branch of the left coronary artery was achieved by tying an 8–0 silk suture around the artery. The suture was passed under the artery at a position ~1 mm from the tip of the normally positioned left auricle.

For studies of the effect of reperfusion after coronary artery occlusion, the ligature was tied

at the same location on the coronary artery used for the permanent occlusion. However, to allow subsequent reestablishment of blood flow, occlusion was produced by placing a 1 mm length of polyethylene (PE) tubing (OD = 0.61 mm) on the artery and fixing it in place with the ligature. The artery was then compressed by tightening the ligature, producing myocardial blanching and electrocardiographic (ECG) S-T segment elevation as observed in permanent ligations. After occlusion for the desired time, blood flow was restored by removing the ligature and PE tubing. The chest wall was then closed by a 6–0 Ticron suture with one layer through the chest wall and muscle and a second layer through the skin and subcutaneous layer.

After surgical closing of the chest, the endotracheal tube was removed, warmth was provided by a heat lamp, and 100 % oxygen was provided via a nasal cone. The animal was given 0.1 mg/kg butorphanol tartrate as an analgesic, and it became sternally recumbent within 1 h. After surviving the experimental infarct, the mice recovered, and this allowed postoperative physiological measurement. Sham-operated mice underwent an identical procedure with placement of the ligature but did not undergo coronary artery occlusion.

Modifications of the Method

Guo et al. (1998) demonstrated the effects of an early and a late phase of ischemic preconditioning in mice. The results demonstrated that, in the mouse, a robust infarct-sparing effect occurred during both the early and the late phases of ischemic preconditioning, although the early phase was more powerful.

Guo et al. (2005) found that late preconditioning induced by NO donors, adenosine A_1 receptor agonists, and δ_1-opioid receptor agonists is mediated by inducible NO synthase.

Lutgens et al. (1999) reported cardiac structural and functional changes after chronic myocardial infarction in the mouse.

Scherrer-Crosbie et al. (1999) described echocardiographic determination of risk area in a murine model of myocardial ischemia. Myocardial contrast echocardiography was performed before and after coronary artery ligation in anesthetized mice by intravenous injection of contrast microbubbles and transthoracic echo imaging. Time–video intensity curves were obtained for the anterior, lateral, and septal myocardial walls. After myocardial ischemia, myocardial contrast echocardiography defects were compared with the area of no perfusion measured by Evans blue staining.

Jones and Lefer (2001) described cardioprotective actions of acute HMG-CoA reductase inhibition in the setting of myocardial infarction.

Janssens et al. (2004) reported that cardiomyocyte-specific overexpression of NO synthase 3 (NOS3) improves left ventricular (LV) performance and reduces compensatory hypertrophy after myocardial infarction. The effect of cardiomyocyte-restricted overexpression of one NO synthase isoform, NOS3, on LV remodeling after myocardial infarction in mice was tested. LV structure and function before and after permanent left anterior descending (LAD) coronary artery ligation were compared in transgenic mice with cardiomyocyte-restricted NOS3 overexpression (NOS3-TG) and their wild-type littermates (WT). Before myocardial infarction, systemic hemodynamic measurements, echocardiographic assessment of LV fractional shortening (FS), heart weight, and myocyte width (as assessed histologically) did not differ in NOS3-TG and WT mice. The inotropic response to graded doses of isoproterenol was significantly reduced in NOS3-TG mice. One week after LAD ligation, the infarcted fraction of the LV did not differ in WT and NOS3-TG mice. Four weeks after myocardial infarction, however, end-systolic LV internal diameter (LVID) was greater, and FS and maximum and minimum rates of LV pressure development were less in WT than in NOS3-TG mice. LV weight/body weight ratio was greater in WT than in NOS3-TG mice.

LaPointe et al. (2004) found that inhibition of cyclooxygenase-2 (COX-2) improves cardiac function after myocardial infarction in the mouse. Myocardial infarction was produced by ligation of the LAD coronary artery in mice. Two days later, mice were treated with a selective COX-2 inhibitor or vehicle in drinking water

for 2 weeks. After the treatment period, mice were subjected to two-dimensional M-mode echocardiography to determine cardiac function. Hearts were then analyzed for the determination of infarct size, interstitial collagen content, brain natriuretic peptide (BNP) mRNA, myocyte cross-sectional area, and immunohistochemical staining for transforming growth factor (TGF)β and COX-2.

Shibuya et al. (2005) reported that *N*-acetyl-seryl-aspartyl-lysine-proline prevents renal insufficiency and matrix expansion in diabetic d*b*/d*b* **mice**.

Weinberg et al. (2005) found in coronary ligation experiments in mice that rosuvastatin reduces experimental left ventricular infarct size after ischemia–reperfusion injury but not total coronary occlusion.

Yang et al. (2005) found that the infarct-sparing effect of A_{2A}-adenosine receptor activation is due primarily to its action on lymphocytes. Chimeric mice were created by bone marrow transplantation from A_{2A}AR-knockout or green fluorescent protein (GFP) donor mice to irradiated congenic C57BL/6 (B6) recipients. In the GFP chimeras, we were unable to detect GFP-producing cells in the vascular endothelium, indicating that bone marrow-derived cells were not recruited to endothelium at appreciable levels after bone marrow transplantation and/or acute myocardial infarction. Injection of 5 or 10 μg/kg of a potent and selective agonist of A_{2A} adenosine receptor had no effect on hemodynamic parameters but reduced infarct size in B6 mice after 45 min of LAD artery occlusion followed by 24 h of reperfusion.

Kanno et al. (2003) found **connexin**43 to be a determinant of myocardial infarct size following coronary occlusion in mice.

Regulation of myocardial connexins during hypertrophic remodeling was reviewed by Teunissen et al. (2004).

Kuhlmann et al. (2006) reported that granulocyte colony-stimulating factor (G-CSF), alone or in combination with stem cell factor (SCF), can improve hemodynamic cardiac function after myocardial infarction in mice and reduces inducible arrhythmias in the infarcted heart potentially via increased connexin43 expression and arteriogenesis.

Gargiulo et al. (2012) discusses the applications of hybrid cardiac positron emission tomography/X-ray computed tomography (PET/CT) systems technology for imaging of mouse models of myocardial infarction.

References and Further Reading

Azizi M, Rousseau A, Ezan E, Guyene TT, Michelet S, Grognet JM, Lefant M, Corvol P, Ménard J (1996) Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator *N*-acetylseryl-aspartyl-lysyl-proline. J Clin Invest 97:839–844

Azizi M, Ezan E, Nicolet L, Grognet JM, Ménard J (1997a) High plasma level of *N*-acetyl-seryl-aspartyl-lysyl-proline. A new marker of chronic angiotensin-converting enzyme inhibition. Hypertension 30:1015–1019

Azizi M, Rousseau A, Ezan E, Guyene TT, Michelet S, Grognet JM, Lenfant M, Corvol P, Menard J (1997b) Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator *N*-acetylseryl-aspartyl-lysyl-proline. J Clin Invest 97:839–844

Bäcklund T, Palojoki E, Saraste A, Eriksson A, Finkenberg P, Kytö V, Lakkisto P, Mervaala E, Voipio-Pulkki LM, Laine M, Tikkanen L (2004) Sustained cardiomyocyte apoptosis and left ventricular remodelling after myocardial infarction in experimental diabetes. Diabetologia 47:325–330

Belichard P, Savard P, Cardinal R, Naddeau R, Gosselin H, Paradis P, Rouleau JL (1994) Markedly different effects on ventricular remodelling result in a decrease in inducibility of ventricular arrhythmias. J Am Coll Cardiol 23:505–513

Bernauer W (1985) The effect of β-adrenoceptor blocking agents on evolving myocardial necrosis in coronary ligated rats with and without reperfusion. Naunyn-Schmiedeberg's Arch Pharmacol 328:288–294

Böhm M, Gierschik P, Jakobs KH, Pieske B, Schnabel P, Ungerer M, Erdmann E (1990)

Increase of $G_{i\alpha}$ in human hearts with dilated but not ischemic cardiomyopathy. Circulation 82:1249–1265

Böhm M, Eschenhagen T, Gierschik P, Larisch K, Lensche H, Mende U, Schmitz W, Schnabel P, Scholz H, Steinfath M, Erdmann H (1994) Radioimmunochemical quantification of $G_{i\alpha}$ in right and left ventricles from patients with ischemic and dilated cardiomyopathy and predominant left ventricular failure. J Mol Cell Cardiol 26:133–149

Chen L, Chen CX, Gan XT, Beier N, Scholz W, Karmazyn M (2004) Inhibition and reversal of myocardial infarction-induced hypertrophy and heart failure by NHE-1 inhibition. Am J Physiol 286:H381–H387

Chiariello M, Brevetti G, DeRosa G, Acunzo F, Petillo F, Rengo F, Condorelli M (1980) Protective effects of simultaneous alpha and beta adrenergic receptor blockade on myocardial cell necrosis after coronary arterial occlusion in rats. Am J Cardiol 46:249–254

Colatsky TH (1989) Models of myocardial ischemia and reperfusion injury: role of inflammatory mediators in determining infarct size. In: Pharmacological methods in the control of inflammation. Alan R. Liss, New York, pp 283–320

Fishbein MC, Hare AC, Gissen SA, Spadaro J, MacLean D, Maroko PR (1980) Identification and quantification of histochemical border zones during the evolution of myocardial infarction in the rat. Cardiovasc Res 14:41–49

Fishbein MC, MacLean D, Maroko PR (1978) Experimental myocardial infarction in the rat. Qualitative and quantitative changes during pathologic evolution. Am J Pathol 90:57–70

Flaim SF, Zelis R (1981) Diltiazem pretreatment reduces experimental myocardial infarct size in rat. Pharmacology 23:281–286

Gargiulo S, Greco A, Gramanzini M, Petretta MP, Ferro A, Larobina M, Panico M, Brunetti A, Cuocolo A (2012) PET/CT imaging in mouse models of myocardial ischemia. J Biomed Biotechnol 2012:541872

Gomoll AW, Lekich RF (1990) Use of the ferret for a myocardial ischemia/salvage model. J Pharmacol Methods 23:213–223

Gould KE, Taffet GE, Michael LH, Christie RM, Konkol DL, Pocius JS, Zachariah JP, Chaupin DF, Daniel SL, Sandusky GE Jr, Hartley CJ, Entman ML (2001) Heart failure and greater infarct expansion in middle-aged mice: a relevant model for postinfarction failure. Am J Physiol 282:H615–H621

Guo Y, Wu WJ, Qiu Y, Tang XL, Yang Z, Bolli R (1998) Demonstration of an early and a late phase of ischemic preconditioning in mice. Am J Physiol 275:H1375–H1387

Guo Y, Stein AB, Wu WJ, Zhu X, Tan W, Li Q, Bolli R (2005) Late preconditioning induced by NO donors, adenosine A_1 receptor agonists, and δ_1-opioid receptor agonists is mediated by iNOS. Am J Physiol 289:H2251–H2257

Innes IR, Weisman H (1981) Reduction in the severity of myocardial infarction by sulfinpyrazone. Am Heart J 102:153–157

Janssens S, Pokreisz P, Schoonjans L, Pellens M, Vermeersch P, Tjwa M, Jans P, Scherrer-Crosbie M, Picard MH, Szelid Z, Gillijns H, Van de Werf F, Collen D, Bloch KD (2004) Cardiomyocyte-specific overexpression of nitric oxide synthase 3 improves left ventricular performance and reduces compensatory hypertrophy after myocardial infarction. Circ Res 94:1256–1262

Johns TNP, Olson BJ (1954) Experimental myocardial infarction. I. A method of by coronary occlusion in small animals. Ann Surg 140:675–682

Jones SP, Lefer DJ (2001) Cardioprotective actions of acute HMG-CoA reductase inhibition in the setting of myocardial infarction. Acta Physiol Scand 173:139–143

Kajstura J, Zhang X, Reiss K, Szoke E, Li P, Lagastra C, Cheng W, Darzynkiewicz Z, Olivetti G, Anversa P (1994) Myocyte cellular hyperplasia and myocyte cellular hypertrophy contribute to chronic ventricular remodeling in coronary artery narrowing-induced cardiomyopathy in rats. Circ Res 74:382–400

Kanno S, Kovacs A, Yamada KA, Saffitz JE (2003) Connexin43 as a determinant of myocardial infarct size following coronary occlusion in mice. J Am Coll Cardiol 41:681–686

Kaufman N, Gavan TL, Hill RW (1959) Experimental myocardial infarction in the rat. Arch Pathol 57:482–488

Kim DY, Kim HJ, Yu KH, Min JJ (2012) Synthesis of 18F-labeled (2-(2-fluoroethoxy)ethyl) triphenylphosphonium cation as a potential agent for myocardial imaging using positron emission tomography. Bioorg Med Chem Lett 22:319–322

Kim DY, Kim HS, Jang HY, Kim JH, Bom HS, Min JJ (2014) Comparison of the cardiac microPET images obtained using [(18)F] FPTP and [(13)N]NH3 in rat myocardial infarction models. ACS Med Chem Lett 5 (10):1124–1128

Kouchi I, Zolk O, Jockenhövel F, Itter G, Linz W, Cremers B, Böhm M (2000) Increase in $G_{i\alpha}$ protein accompanies progression of post-infarction remodelling in hypertensive cardiomyopathy. Hypertension 36:42–47

Kuhlmann MT, Kirchhof P, Klocke R, Hasib L, Stypmann J, Fabritz L, Stelljes M, Tian W, Zwiener M, Mueller M, Kienast J, Breithardt G, Nikol S (2006) G-CSF/SCF reduces inducible arrhythmias in the infracted heart potentially via increased connexin43 expression and arteriogenesis. J Exp Med 203:87–97

LaPointe MC, Mendez M, Leung A, Tao Z, Yang XP (2004) Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse. Am J Physiol 286: H1416–H1424

Le Meur Y, Aldigier JC, Praloran V (1998) Is plasma Ac-SDKP level is a reliable marker of chronic angiotensin-converting enzyme inhibition in hypertensive patients? Hypertension 31:1201–1202

Leprán I, Koltai M, Szekeres L (1981) Effect of non-steroid antiinflammatory drugs in experimental myocardial infarction in rats. Eur J Pharmacol 69:235–238

Linz W, Wiemer G, Schmidts HL, Ulmer W, Ruppert D, Schölkens BA (1996) ACE inhibition decreases postoperative mortality in rats with left ventricular hypertrophy and myocardial infarction. Clin Exp Hypertens 18:691–712

Liu YH, Yang XP, Nass O, Sabbah HN, Peterson E, Carretero OA (1997) Chronic heart failure induced by coronary artery ligation in Lewis inbred rats. Am J Physiol 272 (2 Pt2):H722–H727

Lutgens E, Daemen MJ, de Muinck ED, Debets J, Leenders P, Smits JF (1999) Chronic myocardial infarction in the mouse: cardiac structural and functional changes. Cardiovasc Res 41:586–593

MacLean D, Fishbein MC, Braunwald E, Maroko PR (1978) Long-term preservation of ischemic myocardium after experimental coronary artery occlusion. J Clin Invest 61:541–551

Michael LH, Entman ML, Hartley CJ, Youker KA, Zhu J, Hall SR, Hawkins HK, Berens K, Balantyne CM (1995) Myocardial ischemia and reperfusion: a murine model. Am J Physiol 269(Heart Circ Physiol 38):H2147–H2154

Michael LH, Ballantyne CM, Zachariah JP, Gould KE, Pocius JS, Taffet GE, Hartley CJ, Pham TT, Daniel SL, Funk E, Entman ML (1999) Myocardial infarction and remodeling in mice: effect of reperfusion. Am J Physiol 277: H660–H668

Nguyen T, Salibi EE, Rouleau JL (1998) Postinfarction survival and inducibility of ventricular arrhythmias in the spontaneously hypertensive rat. Effects of ramipril and hydralazine. Circulation 98:2074–2080

Pfeffer JM, Pfeffer MA, Braunwald E (1985) Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ Res 57:84–95

Porzio S, Masseroli M, Messori A, Forloni G, Olivetti G, Jeremic G, Riva E, Luvara G, Latini R (1995) A simple, automatic method for morphometric analysis of the left ventricle in rats with myocardial infarction. J Pharmacol Toxicol Methods 33:221–229

Rasoul S, Carretero OA, Peng H, Cavasin MA, Zhuo J, Sanchez-Mendoza A, Brigstock DR, Rhaleb NE (2004) Antifibrotic effect of Ac-SDKP and angiotensin converting enzyme inhibition in hypertension. J Hypertens 22:593–603

Roberts CS, MacLean D, Braunwald E, Maroko PR, Kloner RA (1983) Topographic changes in

the left ventricle after experimentally induced myocardial infarction in the rat. Am J Cardiol 51:873–876

Sakai K, Akima M, Aono J (1981) Evaluation of drug effects in a new experimental model of angina pectoris in the intact anesthetized rat. J Pharmacol Methods 5:325–336

Scherrer-Crosbie M, Steudel W, Ullrich R, Hunziker PR, Liel-Cohen N, Newell J, Zaroff J, Zapol WM, Picard MH (1999) Echocardiographic determination of risk area in a murine model of myocardial ischemia. Am J Physiol 277:H986–H992

Scholz W, Albus U, Counillon L, Gögelein H, Lang HJ, Linz W, Weichert A, Schölkens BA (1995) Protective effects of HOE 642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischemia and reperfusion. Cardiovasc Res 29:260–268

Selye H, Bajusz E, Grasso S, Mendell P (1960) Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 11:398–407

Shibuya K, Kanasaki K, Isono M, Sato H, Omata M, Sugimoto T, Araki SI, Isshiki K, Kashiwagi A, Haneda M, Koya D (2005) *N*-Acetyl-seryl-aspartyl-lysine-proline prevents renal insufficiency and matrix expansion in diabetic d*b*/d*b* mice. Diabetes 54:838–845

Sia YT, Lapointe N, Parker TG, Tsoporis JN, Deschepper CP, Calderose A, Pourdjabbar A, Jasmin JF, Sarrazin JF, Liu P, Asam A, Butany J, Rouleau JL (2002) Beneficial effects of long-term use of the antioxidant probucol in heart failure in the rat. Circulation 105:2549–2555

Teunissen BEJ, Jongsma HJ, Bierhuizen MFA (2004) Regulation of myocardial connexins during hypertrophic remodelling. Eur Heart J 25:1979–1989

Walker MJA, MacLeod BA, Curtis MJ (1991) Myocardial ischemia and infarction. Comp Pathol Bull 23:3–4

Weinberg EO, Scherrer-Crosbie M, Picard MH, Nasseri BA, MacGillivray C, Gannon J, Lian Q, Bloch KD, Lee RT (2005) Rosuvastatin reduces experimental left ventricular infarct size after ischemia-reperfusion injury but not total coronary occlusion. Am J Physiol 288:H1802–H1809

Yang XP, Sabbah HN, Liu YH, Sharov VG, Mascha EJ, Alwan I, Carretero OA (1992) Ventriculographic evaluation in three rat models of cardiac dysfunction. Am J Physiol 265(6 Pt 2):H1946–H1952

Yang F, Yang XP, Liu YH, Xu J, Cingolani O, Rhaleb NE, Carretero OA (2004) Ac-SDKP reverses inflammation and fibrosis in rats with heart failure after myocardial infarction. Hypertension 43:229–236

Yang Z, Day YJ, Toufektsian MC, Ramos SI, Marshall M, Wang XQ, French BA, Linden J (2005) Infarct-sparing effect of A_{2A}-adenosine receptor activation is due primarily to its action on lymphocytes. Circulation 111:2190–2197

Ytrehus K, Liu Y, Tsuchida A, Miura T, Liu GS, Yang XM, Herbert D, Cohen MV, Downey JM (1994) Rat and rabbit heart infarction: effects of anesthesia, perfusate, risk zone, and method of infarct sizing. Am J Physiol 267(Heart Circ Physiol 36):H2383–H2390

Occlusion of Coronary Artery in Anesthetized Dogs and Pigs

Purpose and Rationale

The size of infarcts is studied after proximal occlusion of the left anterior descending coronary artery in open chest dogs. Compounds potentially reducing infarct size are tested. To delineate the postmortem area at risk, coronary arteriograms are made after injection of a $BaSO_4$-gelatin mass into the left coronary ostium. The infarct's area is visualized with nitroblue tetrazolium chloride in myocardial sections.

Procedure

Dogs of either sex weighing approximately 30 kg are used. The animals are anesthetized by intravenous injection of pentobarbital sodium (bolus of 35 mg/kg followed by continuous infusion of 4 mg/kg/h). The animals are placed in the right lateral position. Respiration is maintained through a tracheal tube using a positive pressure respirator.

Arterial blood gases are checked, and the ventilation rate and/or oxygen flow rate is adjusted to achieve physiological blood gas values (P_{O2}: 100–140 mmHg, P_{CO2}: 32–40 mmHg, and pH7.47). A peripheral vein (saphenous vein) is cannulated for the administration of test compound. The ECG is recorded continuously from lead II (Einthoven).

Preparation for Hemodynamic Measurements

For recording of peripheral systolic and diastolic blood pressure, the cannula of a femoral vein is connected to a pressure transducer (Statham P 23DB). For the determination of left ventricular pressure (LVP), a Millar microtip catheter (PC350) is inserted via the left carotid artery. Left ventricular end-diastolic pressure (LVEDP) is measured from a high-sensitivity scale. From the pressure curve, dp/dt max is differentiated and heart rate is counted.

Experimental Procedure

The heart is exposed through a left thoracotomy between the fourth and fifth intercostal space, the pericard is opened, and the left anterior descending coronary artery (LAD) is exposed. After reaching steady-state conditions for the hemodynamic parameters (approx. 45 min), the LAD is ligated just below the first diagonal branch for 360 min. No attempt is made to suppress arrhythmic activity after the ligation.

The test substance or the vehicle (controls) is administered by intravenous bolus injection and/or continuous infusion. The schedule of administration may vary. Hemodynamic parameters are registered continuously during the whole experiment. At the end of the experiment, the animals are sacrificed with an overdose of pentobarbital sodium and the heart is dissected.

Preparation to Determine Area at Risk

Coronary arteriograms are made according to Schaper et al. (1979) to delineate the anatomic postmortem area at risk. A purse-string suture is placed around the left coronary ostium in the sinus of Valsalva; a cannula is then placed in the ostium and the purse-string suture is tightened. Micronized $BaSO_4$ suspended in 12 % gelatin solution (37 °C) is injected under increasing pressure (2 min at 100 mmHg, 2 min at 150 mmHg, and 2 min at 200 mmHg). The heart is placed in crushed ice to gel the injectate. The right ventricle is removed, and the left ventricle plus septum is cut into transverse sections (approx. 1 cm thick) from the apex to the level of the occlusion (near the base). From each slice, angiograms are made with an X-ray tube at 40 kV to assess the postmortem area at risk (by defect opacity: reduction of $BaSO_4$-filled vessels in infarct tissue).

Preparation to Determine Infarct Size

The slices are then incubated in p-nitroblue tetrazolium solution (0.25 g/L in Sörensen' phosphate buffer, pH7.4, containing 100 mM D, L-maleate) in order to visualize the infarct tissue (blue-/violet-stained healthy tissue, unstained necrotic tissue). The slices are photographed on color transparency film for the determination of the infarct area.

Left ventricle and infarct area and area at risk are measured by planimetry from projections of all slices with the exclusion of the apex and of the slice containing the ligature.

Evaluation

Mortality and the different hemodynamic parameters are determined. Changes of parameters in drug-treated animals are compared to vehicle controls. The different characteristics are evaluated separately. Mean values ± SEM of infarct area and of area at risk are calculated. Statistical analyses consist of regression and correlation analyses and of the Student's t-test. Results are considered significant at $p < 0.05$.

Modifications of the Method

Nachlas and Shnitka (1963) described the macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity in dogs by staining the cardiac tissue with nitro-BT [2,2′-di-p-nitrophenyl-5,5′ diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene) ditetrazolium chloride] yielding a dark blue formazan in viable muscle but not in necrotic muscle fibers.

Chiariello et al. (1976) compared the effects of nitroprusside and nitroglycerin on ischemic injury during acute myocardial infarction in dogs.

Black et al. (1995) studied the cardioprotective effects of heparin or *N*-acetylheparin in an in vivo dog model of myocardial and ischemic reperfusion injury. The hearts were removed after 90 min of coronary occlusion and a 6 h reperfusion period. Area at risk was determined by the absence of Evans blue dye after perfusion of the aorta in a retrograde fashion and infarct zone by the absence of formazan pigment within the area at risk after perfusion of the circumflex coronary artery with triphenyltetrazolium chloride.

Reimer et al. (1985) tested the effect of drugs to protect ischemic myocardium in unconscious and conscious dogs. In the conscious model, dogs of either sex weighing 10–25 kg were anesthetized with thiamylal sodium (30–40 mg/kg i.v.) and underwent thoracotomy through the 4th intercostal space. Heparin-filled polyvinyl chloride catheters were positioned in the aortic root, the left atrium via the left atrial appendage, and a systemic vein. A mechanical adjustable snare-type occluder was placed around the proximal left circumflex coronary artery above or below the first marginal branch, so that temporary occlusion resulted in cyanosis of at least 75 % of the inferior wall. The catheters and snare were either exteriorized or positioned in a subcutaneous pocket at the back of the neck. Penicillin, 1,000,000 units, and streptomycin, 1.0 g, were given i.m. for the first 4 postoperative days, and at least 7 days were allowed for recovery from surgery.

Dogs were fasted overnight prior to the study. After exteriorization and flushing of the catheters, 30–40 min were allowed for the animals to adjust to laboratory conditions. Morphine sulfate, 0.25 mg/kg, i.m., was given 30 min before occlusion, and an additional 0.25 mg/kg, i.v., was given 20 min later. Heart rate and aortic and left atrial pressures were monitored continuously. Permanent coronary occlusion was produced by a sudden one-stage tightening of the snare occluder. Drugs were administered by continuous i.v. infusion over 6 h. Hemodynamic measurements were taken 5 min before occlusion and 10, 25, 105, 180, and 360 min after occlusion.

Raberger et al. (1986) described a model of **transient myocardial dysfunction in conscious dogs**. Mongrel dogs, trained to run on a treadmill, were chronically instrumented with a miniature pressure transducer in the left ventricle, and a hydraulic occluder was placed around the circumflex branch of the left coronary artery. Two pairs of piecoelectrical crystals for sonomicrometry were implanted subendocardially to measure regional myocardial functions. Comparable episodes of regional dysfunction of the left coronary artery area during treadmill runs were found after partial left coronary artery stenosis induced by external filling of the occluder.

Hartman and Warltier (1990) described a model of **multivessel coronary artery disease** using conscious, chronically instrumented dogs. A hydraulic occluder was implanted around the left anterior descending coronary artery (LAD) and an ameroid constrictor around the left circumflex coronary artery (LCCA). Pairs of piecoelectric crystals were implanted within the subendocardium of the LAD and LCCA perfusion territories to measure regional contractile function. A catheter was placed in the left atrial appendage for injection of radioactive microspheres to measure regional myocardial perfusion. Bolus injections of adenosine were administered daily via the left atrium to evaluate LAD and LCCA coronary reserve. After stenosis by the ameroid constrictor, radioactive microspheres were administered to compare regional perfusion within normal myocardium to flow in myocardium supplied by the occluded or stenotic coronary arteries.

Holmborn et al. (1993) compared triphenyltetrazolium chloride staining versus detection of fibronectin in experimental myocardial infarction in **pigs**.

Klein et al. (1995) used intact **pigs** and found myocardial protection by Na^+/H^+ exchange inhibition in ischemic–reperfused hearts.

Klein et al. (1997) measured the time delay of cell death by Na^+/H^+ exchange inhibition in regionally ischemic–reperfused **porcine** hearts.

Garcia-Dorado et al. (1997) determined the effect of Na^+/H^+ exchange blockade in ischemic rigor contracture and reperfusion-induced

hypercontracture in *pigs* submitted to 55 min of coronary occlusion and 5 h reperfusion. Myocardial segment length analysis with ultrasonic microcrystals was used to detect ischemic rigor (reduction in passive segment length change) and hypercontracture (reduction in end-diastolic length).

Symons et al. (1998) tested the attenuation of regional dysfunction in response to 25 cycles of ischemia (2 min) and reperfusion (8 min) of the left circumflex coronary artery in **conscious swine** after administration of a Na^+/H^+ exchange inhibitor. The animals were instrumented to measure arterial blood pressure, regional myocardial blood flow (colored microspheres), systolic wall thickening in the normally perfused left anterior descending and left circumflex coronary artery regions (sonomicrometry), left circumflex coronary artery blood flow velocity (Doppler), and reversibility to occlude the left circumflex coronary artery (hydraulic occluder).

Etoh et al. (2001) studied myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in **pigs**.

McCall et al. (2012) described a model of myocardial infarction using Yorkshire and Göttingen swines. Myocardial infarction was created with a closed-chest coronary artery occlusion–reperfusion and encompasses the anteroapical, lateral, and septal walls of the left ventricle.

Lukács et al. (2012) reviewed the small and large animal models of experimental myocardial infarction regarding the differences among species, methods, reproducibility, and interpretation.

Lukács et al. (2013) evaluated the electromechanical mapping diagnostic in parallel with cardiac magnetic resonance imaging tool in porcine myocardial infarction models (balloon occlusion in the left anterior descending coronary artery or coil deployment in the LAD or circumflex artery).

Li et al. (2013) showed that prophylactic use of amiodarone plus lidocaine reduces arrhythmia and mortality after acute myocardial infarction in sheep model without significant negative effect on hemodynamics. This improved cost and acceptance of a large myocardial infarction animal models. Spata et al. (2013) reported an acute myocardial infarction model in sheep created by catheter injection of autologous aggregated platelets into the mid-left anterior descending coronary artery.

References and Further Reading

Abendroth RR, Meesmann W, Stephan K, Schley G, Hübner H (1977) Effects of the β-blocking agent Atenolol on arrhythmias especially ventricular fibrillation and fibrillation threshold after acute experimental coronary artery occlusion. Z Kardiol 66:341–350

Black SC, Gralinski MR, Friedrichs GS, Kilgore KS, Drsicoll EM, Lucchesi BR (1995) Cardioprotective effects of heparin or *N*-acetylheparin in an *in vivo* model of myocardial and ischemic reperfusion injury. Cardiovasc Res 29:629–636

Chiariello M, Gold HL, Leinbach RC, Davis MA, Maroko PR (1976) Comparison between the effects of nitroprusside and nitroglycerin on ischemic injury during acute myocardial infarction. Circulation 54:766–773

Etoh T, Joffs C, Deschamps AM, Davis J, Dowdy K, Hendrick J, Baicu S, Mukherjee R, Manhaini M, Spinale FG (2001) Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs. Am J Physiol 281:H987–H994

Garcia-Dorado D, Ganzález MA, Barrabés JA, Ruiz-Meana M, Solares J, Lidon RM, Blanco J, Puigfel Y, Piper HM, Soler-Soler J (1997) Prevention of ischemic rigor contracture during coronary occlusion by inhibition of Na^+-H^+ exchange. Cardiovasc Res 35:80–89

Hartman JC, Warltier DC (1990) A model of multivessel coronary artery disease using conscious, chronically instrumented dogs. J Pharmacol Methods 24:297–310

Holmborn B, Näslund U, Eriksson A, Virtanen I, Thornell LE (1993) Comparison of triphenyltetrazolium chloride (TTC) staining versus detection of fibronectin in experimental myocardial infarction. Histochemistry 99:265–275

Klein HH, Pich S, Bohle RM, Wollenweber J, Nebendahl K (1995) Myocardial protection by Na^+/H^+ exchange inhibition in ischemic, reperfused porcine hearts. Circulation 92:912–917

Klein HH, Bohle RM, Pich S, Lindert-Heimberg S, Wollenweber J, Nebendahl K (1997) Time delay of cell death by Na^+/H^+ exchange inhibition in regionally ischemic, reperfused porcine hearts. J Cardiovasc Pharmacol 30:235–240

Li T, Wei X, Watkins AC, Sanchez PG, Wu ZJ, Griffith BP (2013) Prophylactic amiodarone and lidocaine improve survival in an ovine model of large size myocardial infarction. J Surg Res 185(1):152–158

Lukács E, Magyari B, Tóth L, Petrási Z, Repa I, Koller A, Horváth I (2012) Overview of large animal myocardial infarction models. Acta Physiol Hung 99(4):365–381

Lukács E, Magyari B, Tóth L, Petneházy Ö, Petrási Z, Simor T, Gyöngyösi M, Repa I, Koller Á, Rőth E, Horváth IG (2013) Evaluation of experimental myocardial infarction models via electromechanical mapping and magnetic resonance imaging. Can J Physiol Pharmacol 91(8):617–624

Martorana PA, Göbel H, Kettenbach B, Nitz RE (1982) Comparison of various methods for assessing infarct-size in the dog. Basic Res Cardiol 77:301–308

Martorana PA, Kettenbach B, Breipohl G, Linz W (1990) Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a bradykinin antagonist. Eur J Pharmacol 182:395–396

McCall FC, Telukuntla KS, Karantalis V, Suncion VY, Heldman AW, Mushtaq M, Williams AR, Hare JM (2012) Myocardial infarction and intramyocardial injection models in swine. Nat Protoc 7(8):1479–1496

Nachlas MN, Shnitka TK (1963) Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. Am J Pathol 42:379–396

Raberger G, Krumpl G, Mayer N (1986) A model of transient myocardial dysfunction in conscious dogs. J Pharmacol Methods 16:23–37

Reimer KA, Jennings RB, Cobb FR, Murdock RH, Greenfield JC, Becker LC, Bulkley BH, Hutchins GM, Schwartz RP, Bailey KR, Passamani ER (1985) Animal models for protecting ischemic myocardium: results of the NHLBI cooperative study. Comparison of unconscious and conscious dog models. Circ Res 56:651–665

Schaper W, Frenzel H, Hort W (1979) Experimental coronary artery occlusion. I. Measurement of infarct size. Basic Res Cardiol 74:46–53

Scherlag BJ, El-Sherif N, Hope R, Lazzara R (1974) Characterization and localization of ventricular arrhythmias resulting from myocardial ischemia and infarction. Circ Res 35:372–383

Spata T, Bobek D, Whitson BA, Parthasarathy S, Mohler PJ, Higgins RS, Kilic A (2013) A nonthoracotomy myocardial infarction model in an ovine using autologous platelets. Biomed Res Int 2013:938047

Symons JD, Correa SD, Schaefer S (1998) Na-H exchange inhibition with cariporide limits functional impairment due to repetitive ischemia. J Cardiovasc Pharmacol 32(6):853–862

Acute Ischemia by Injection of Microspheres in Dogs

Purpose and Rationale

Severe left ventricular failure is induced by repeated injections of 50 μm plastic microspheres into the left main coronary artery of anesthetized dogs. Hemodynamic measurements are performed under these conditions, testing drugs which potentially improve cardiac performance. The test can be used to evaluate the influence of drugs on myocardial performance during acute ischemic left ventricular failure in dogs.

Procedure

Dogs of either sex weighing approximately 30 kg are anesthetized by an intravenous bolus injection of 35–40 mg/kg pentobarbital sodium continued by an infusion of 4 mg/kg/h. The animals are placed in the right lateral position. Respiration is maintained through a tracheal tube using a positive pressure respirator and controlled by measuring end-expiratory CO_2 concentration as well as blood gases. Two peripheral veins are

cannulated for the administration of narcotic (brachial vein) and test compounds (saphenous vein). The ECG is recorded continuously in lead II (Einthoven).

Preparation for Hemodynamic Measurements

For recording of peripheral systolic and diastolic blood pressure, the cannula of the right femoral vein is connected to a pressure transducer (Statham P 23DB). For the determination of left ventricular pressure (LVP), a Millar microtip catheter (Gould PC 350) is inserted via the left carotid artery. Left ventricular end-diastolic pressure (LVEDP) is measured on a high-sensitivity scale. From the pressure curve, dp/dt max is differentiated and heart rate (HR) is counted. To measure right ventricular pressure, a Millar microtip catheter is inserted via the right femoral vein. Systolic, diastolic, and mean pulmonary artery pressure (PAP), mean pulmonary capillary pressure, and cardiac output are measured by a thermodilution technique using a cardiac index computer (Gould SP 1435) and a balloon tip triple lumen catheter (Gould SP 5105, 5F) with the thermistor positioned in the pulmonary artery via the jugular vein.

The heart is exposed through a left thoracotomy between the fourth and fifth intercostal space, the pericard is opened, and the left circumflex coronary artery (LCX) is exposed. To measure coronary blood flow, an electromagnetic flow probe (Hellige Recomed) is placed on the proximal part of the LCX.

Polystyrol microspheres (3 M Company, St. Paul, Minnesota, USA) with a diameter of 52.5 ± 2.24 μm are diluted with dextran 70, 60 mg/ml and saline at a concentration of 1 mg microspheres/ml (1 mg = approx. 12,000 beads). For administration of microspheres, an angiogram catheter (Judkins-Schmidt Femoral-Torque, William Cook, Europe Aps.BP 7) is inserted into the left ostium via the left femoral artery.

Induction of Failure

The microspheres are injected through the angiogram catheter into the left ostium initially as 10 ml and later as 5 ml boluses about 5 min apart. The microsphere injections produce stepwise elevations of LVEDP. Embolization is terminated when LVEDP has increased to 16–18 mmHg and/or PAPm has increased to 20 mmHg and/or heart rate has reached 200 beats/min. The embolization is completed in about 70 min and by injection of an average dose of 3–5 mg/kg microspheres. Hemodynamic variables are allowed to stabilize after coronary embolization for at least 30 min.

Experimental Course

The test substance or the vehicle (controls) is then administered by intravenous bolus injection or continuous infusion or by intraduodenal application.

Recordings are obtained:

- Before embolization.
- After embolization.
- Before administration of test compound.
- 5, 30, 45, 60, 90, 120 and, eventually, 150 and 180 min following administration of test drug. At the end of the experiment, the animal is sacrificed by an overdose of pentobarbital sodium.

Evaluation

Besides the different directly measured hemodynamic parameters, the following data are calculated according to the respective formula:

Stroke volume [ml/s], $SV = \frac{\text{cardiac output}}{\text{heart rate}}$

Tension index [mmHgs], $IT = \frac{BPs \times \text{heart rate}}{1,000}$

Coronary vascular resistance [mmHg min/ml], $CVR = \frac{BPm \times RAPm}{CBF}$

Total peripheral resistance [dyns/cm^5], $TPR = \frac{BPm \times RAPm}{\text{cardiac output}} \times 79.9$

Pulmonary artery resistance [dyns/cm^5], $PAR = \frac{PAPm - PCPm}{\text{cardiac output}} \times 79.9$

Right ventricle work [kg m/min], $RVW = (PAPm - RAPm) \times \text{cardiac output} \times 0.0136$

Left ventricle work [kg m/min], $LVW = (BPm - LVEDP) \times \text{cardiac output} \times 0.0136$

Left ventricular myocardial oxygen consumption

$$[\text{ml } O_2/\text{min}/100\text{ g}],\ MVO_2 = K_1(BPs \times HR) + K_2 \frac{(0.8BPs + 0.2BPd) \times HR \times SV}{BW} + 1.43$$

$K_1 = 4.08 \times 10^{-4}$
$K_2 = 3.25 \times 10^{-4}$
BPs = systolic blood pressure [mmHg]
BPd = diastolic blood pressure [mmHg]
BPm = mean blood pressure [mmHg]
CBF = coronary blood flow in left circumflex coronary artery [ml/min]
$RAPm$ = mean right atrial pressure [mmHg]
$PAPm$ = mean blood pressure of the A. pulmonalis [mmHg]
$PCPm$ = mean pulmonary capillary pressure
HR = heart rate [beats/min]
SV = stroke volume [ml]
BW = body weight [kg]

Changes of parameters in drug-treated animals are compared to vehicle controls; statistical significance of the differences is calculated with the Student's *t*-test.

The mean embolization times, doses of microspheres, and number of microsphere applications are evaluated.

Modifications of the Method

Gorodetskaya et al. (1990) described a simple method to produce acute heart failure by coronary vessel embolization with microspheres in rats.

References and Further Reading

Gorodetskaya EA, Dugin SF, Medvedev OS, Allabergenova AE (1990) A simple method to produce acute heart failure by coronary vessel embolization in closed chest rats with microspheres. J Pharmacol Methods 24:43–51

Rooke GA, Feigl EO (1982) Work as a correlate of canine left ventricular oxygen consumption, and the problem of catecholamine oxygen wasting. Circ Res 50:273–286

Schölkens BA, Martorana PA, Göbel H, Gehring D (1986) Cardiovascular effects of the converting enzyme inhibitor ramipril (Hoe 498) in anesthetized dogs with acute ischemic left ventricular failure. Clin Exp Theory Pract A8(6):1033–1048

Smiseth OA (1983) Effects of the β-adrenergic receptor agonist pirbuterol on cardiac performance during acute ischaemic left ventricular failure in dogs. Eur J Pharmacol 87:379–386

Smiseth OA, Mjøs OD (1982) A reproducible and stable model of acute ischemic left ventricular failure in dogs. Clin Physiol 2:225–239

Influence on Myocardial Preconditioning

Purpose and Rationale

Damage to the mammalian heart produced by prolonged ischemia and reperfusion can be reduced by "preconditioning" the myocardium via a brief cycle of ischemia and reperfusion prior to the protracted ischemic event. Ischemic preconditioning has been shown to decrease infarct size and increase recovery of postischemic ventricular function (Murry et al. 1986) and to reduce leakage of cellular marker proteins indicative for cardiac myocyte death (Volovsek et al. 1992). In addition, preliminary preconditioning also attenuates cardiac arrhythmia associated with subsequent occlusion and reperfusion (Vegh et al. 1990).

The mechanistic basis of this phenomenon is under discussion (Parratt 1994; Parratt and Vegh 1994). Adenosine receptor involvement in myocardial protection after ischemic preconditioning in rabbits has been shown by Baxter et al. (1994). Adenosine (A_1 receptor) antagonists have been demonstrated to block the protection produced by preconditioning (Liu et al. 1991), and short-term administration of adenosine was shown to simulate the protective effects of ischemic preconditioning (Toombs et al. 1993). These observations together suggest that adenosine is generated by the short preconditioning ischemia. Other recent pharmacological studies (Gross and Auchampach 1992; Yao and Gross 1994) indicate the involvement of the ATP-sensitive potassium channel. Recent investigations indicate that an increase of NO production after ACE inhibitors

may be a part of the protective mechanism (Linz et al. 1992, 1994). Moreover, the involvement of prostanoids and bradykinin in the preconditioning process has been discussed (Wiemer et al. 1991). Gho et al. (1994) found a limitation of myocardial infarct size in the rat by transient renal ischemia, supporting the hypothesis that the mechanism leading to cardiac protection by ischemic preconditioning may not only reside in the heart itself.

Procedure

New Zealand rabbits of either sex weighing 2.5–3.5 kg are initially anesthetized with an intramuscular injection of ketamine (50 mg/ml)/xylazine (10 mg/ml) solution at a dose of 0.6 ml per kg body weight. A tracheotomy is performed to facilitate artificial respiration. The left external jugular vein is cannulated to permit a constant infusion (0.15–0.25 ml/min) of xylazine (2 mg/ml in heparinized saline) to assist in maintaining anesthesia and fluid volume. Anesthesia is also maintained by i.m. injections (0.4–0.6 ml) of ketamine (80 mg/ml) and xylazine (5 mg/ml) solution. After the xylazine infusion is started, animals are respired with room air at a tidal volume of 10 ml/kg and a frequency of 30 inflations per min (Harvard Apparatus, USA). Thereafter, ventilation is adjusted or inspiratory room air is supplemented (5 % CO_2/95 % O_2) to maintain arterial blood chemistry within the following ranges: pH 7.35–7.45, P_{CO2} 25–45 mmHg, and P_{O2} 90–135 mmHg. The right femoral artery and vein are isolated and catheterized for measurement of arterial pressure and administration of drugs, respectively.

A thoracotomy is performed in the fourth intercostal space, and the lungs are retracted to expose the heart. The pericardium is cut to expose the left ventricle, and a solid-state pressure transducer catheter (e. g., MicroTip 3F, Millar Instruments, Houston, USA) is inserted through an apical incision and secured to enable measurement of pulsatile left ventricular pressure. The maximal rate of increase in left ventricular pressure (LVd*P*/d*t* max) is determined by electronic differentiation of the left ventricular pressure wave form. A segment of 4–0 prolene suture is looped loosely around a marginal branch of the left main coronary artery to facilitate coronary occlusion during the experiment. Needle electrodes are inserted subcutaneously in a lead II configuration to enable recording of an ECG in order to determine heart rate and help confirm the occurrence of ischemia (ST segment elevation) and reperfusion of the myocardium distal to the coronary occlusion. Continuous recording of pulsatile pressure, ECG, heart rate, and LVd*P*/d*t* is simultaneously displayed on a polygraph (e.g., Gould chart recorder, Gould Inc., Valley View, USA) and digitized in real time by a personal computer. Hemodynamic data are condensed for summary and later statistical analysis.

Ischemic preconditioning is induced by tightening the prolene loop around the coronary artery for 5 min and then loosening to reperfuse the affected myocardium for 10 min prior to a subsequent 30 min occlusion. After surgical preparation, and prior to 30 min of occlusion, rabbits are randomly selected to receive ischemic preconditioning, no preconditioning, or ischemic preconditioning plus treatment with test drugs. After 30 min of occlusion, the ligature is released and followed by 120 min of reperfusion. Occlusion is verified by epicardial cyanosis distal to the suture, which is usually accompanied by alterations in hemodynamics and ECG. Reperfusion is validated by return of original color. Systemic hemodynamics are summarized for each experimental period. The experiment is terminated after 120 min of reperfusion, and the heart is excised for determinations of infarct size and area at risk.

Immediately before the animal is sacrificed, the marginal branch of the left coronary artery is reoccluded, and India ink is rapidly injected by syringe with an 18 g needle into the left ventricular chamber to demarcate blackened normal myocardium from unstained area at risk. After the rabbits are sacrificed, the heart is removed and sectioned in a breadloaf fashion from apex to base perpendicular to the long axis. The right ventricle is removed from each slice leaving only the left ventricle and septum. After each slice is weighed, the portions are washed and incubated in a phosphate buffered saline solution

of triphenyl tetrazolium chloride (1 g/ml, Sigma) for 10–15 min. Salvaged myocardium in the area at risk stains brick red, whereas infarcted tissue remains unaltered in color. Slices are then placed between sheets of Plexiglas and the areas (normal, risk, infarct) of each slice are traced on a sheet of clear acetate. Traces are then digitized and analyzed using computerized planimetry to compare the relative composition of each slice with respect to normal tissue, area at risk, and infarcted myocardium. Planimetry is performed with a computerized analysis system, e.g., Quantimet 570C image analysis system (Leica, Deerfield, USA).

Surface areas of normal tissue, area at risk, and infarcted myocardium on both sides of each slide are averaged for the individual slide. The contribution of each slide to the total infarcted and area at risk (%) and area at risk as a percentage of total left ventricular mass for the entire left ventricle is prorated by the weight of each slice (Garcia-Dorado et al. 1987). By adding the adjusted contributions from each slice to infarcted tissue, area at risk, and left ventricular mass, a three-dimensional mathematical representation of the total myocardial infarct size and risk zone can be calculated for each rabbit and a mean tabulated for each treatment group for statistical comparison.

Evaluation

All data are presented as mean ± SD. Systemic hemodynamic data are analyzed by ANOVA using Statistica/W software. Means are considered significantly different at $p < 0.05$.

Modifications of the Method

Li et al. (1990) found in dog experiments that preconditioning with one brief ischemic interval is as effective as preconditioning with multiple ischemic periods.

In contrast, Vegh et al. (1990) found in other dog experiments that two brief preconditioning periods of coronary occlusion, with an adequate period of reperfusion between, reduce the severity of arrhythmias.

Yang et al. (1996) found a second window of protection after ischemic preconditioning in conscious rabbits which minimizes both infarction and arrhythmias.

Late preconditioning against myocardial stunning in conscious pigs together with an increase of heat stress protein (HSP) 70 was described Sun et al. (1995).

Szilvássy et al. (1994) described the anti-ischemic effect induced by ventricular overdrive pacing as a conscious rabbit model of preconditioning. Rabbits were equipped with right ventricular electrode catheters for pacing and intracavital recording and polyethylene cannulae in the left ventricle and right carotid artery to measure intraventricular pressure and blood pressure. One week after surgery in conscious animals, ventricular overdrive pacing at 500 beats/min over 2, 5, or 10 min resulted in an intracavital S-T segment elevation, shortening of ventricular effective refractory period, decrease in maximum rate of pressure development and blood pressure, and increase in left ventricular end-diastolic pressure proportional to the duration of stimulus. A 5 min preconditioning ventricular overdrive pacing applied 5 or 30 min before a 10 min ventricular overdrive pacing markedly attenuated ischemic changes, whereas a 2 min ventricular overdrive pacing had no effect.

The ventricular overdrive pacing-induced preconditioning effect was lost in atherosclerotic rabbits (Szilvássy et al. 1995); however, delayed cardiac protection could be induced in these animals (Szekeres et al. 1997).

Kharbanda et al. (2002) used an experimental model of myocardial infarction in pigs to demonstrate transient ischemia of the hind limb using a tourniquet to reduce myocardial IR injury. Others (Shimizu et al. 2009) have shown that plasma dialysate from preconditioned animals provides potent cardioprotection of naïve hearts in Langendorff preparation.

Merlocco et al. (2014) described a novel method for inducing cardioprotection by transcutaneous electrical nerve stimulation (TENS). Rabbits were subjected to lower limb TENS, and cardioprotection was evaluated in a Langendorff preparation. TENS reduced infarct size and improved functional recovery during reperfusion.

References and Further Reading

Baxter GF, Marber MS, Patel VC, Yellon DM (1994) Adenosine receptor involvement in a delayed phase of myocardial protection 24 h after ischemic preconditioning. Circulation 90:2993–3000

Garcia-Dorado D, Théroux P, Elizaga J, Galiñanes M, Solares J, Riesgo M, Gomez MJ, Garcia-Dorado A, Aviles FF (1987) Myocardial reperfusion in the pig heart model: infarct size and duration of coronary occlusion. Cardiovasc Res 21:537–544

Gho BC, Schoemaker RG, van der Lee C, Sharma HS, Verdouw PC (1994) Myocardial infarct size limitation in rat by transient renal ischemia. Circulation 90:I-476/2557

Gross JG, Auchampach JA (1992) Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ Res 70:223–233

Hoff PT, Tamura Y, Lucchesi BR (1990) Cardioprotective effects of amlodipine on ischemia and reperfusion in two experimental models. Am J Cardiol 66:10H–16H

Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, Vogel M, Sorensen K, Redington AN, MacAllister R (2002) Transient limb ischemia induces remote ischemic preconditioning in vivo. Circulation 106:2881–2883

Li GC, Vasquez JA, Gallagher KP, Lucchesi BR (1990) Myocardial protection with preconditioning. Circulation 82:609–619

Linz W, Wiemer G, Schölkens BA (1992) ACE-inhibition induces NO-formation in cultured bovine endothelial cells and protects isolated ischemic rat hearts. J Mol Cell Cardiol 24:909–919

Linz W, Wiemer G, Gohlke P, Unger T, Schölkens BA (1994) Kardioprotektive Effekte durch Ramipril nach Ischämie und Reperfusion in tierexperimentellen Studien. Z Kardiol 83 (Suppl 4):53–56

Liu GS, Thornton J, Van Winkle DM, Stanley WH, Olsson RA, Downey JM (1991) Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 84:350–356

Merlocco AC, Redington KL, Disenhouse T, Strantzas SC, Gladstone R, Wei C, Tropak MB, Manlhiot C, Li J, Redington AN (2014) Transcutaneous electrical nerve stimulation as a novel method of remote preconditioning: in vitro validation in an animal model and first human observations. Basic Res Cardiol 109(3):406

Mickelson JK, Simpson PJ, Lucchesi BR (1989) Streptokinase improves reperfusion blood flow after coronary artery occlusion. Int J Cardiol 23:373–384

Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischaemia: a delay of lethal cell injury in ischaemic myocardium. Circulation 74:1124–1136

Parratt JR (1994) Protection of the heart by ischemic preconditioning: mechanisms and possibilities for pharmacological exploitation. TIPS 15:19–25

Parratt J, Vegh A (1994) Pronounced antiarrhythmic effects of ischemic preconditioning. Cardioscience 5:9–18

Shimizu M, Tropak M, Diaz RJ, Suto F, Surendra H, Kuzmin E, Li J, Gross G, Wilson GJ, Callahan J, Redington AN (2009) Transient limb ischaemia remotely preconditions through a humoral mechanism acting directly on the myocardium: evidence suggesting cross-species protection. Clin Sci (Lond) 117:191–200

Simpson PJ, Fantone JC, Mickelson JK, Gallagher KP, Lucchesi BR (1988) Identification of a time window for therapy to reduce experimental canine myocardial injury: suppression of neutrophil activation during 72 h of reperfusion. Circ Res 63:1070–1079

Sun JZ, Tang XL, Knowton AA, Park SW, Qiu Y, Bolli R (1995) Late preconditioning against myocardial stunning. An endogenous protective mechanism that confers resistance to post-ischemic dysfunction 24 h after brief ischemia in conscious pigs. J Clin Invest 95:388–403

Szekeres L, Szilvássy Z, Fernandy P, Nagy I, Karcsu S, Scáti S (1997) Delayed cardiac protection against harmful consequences of stress can be induced in experimental atherosclerosis in rabbits. J Mol Cell Cardiol 29:1977–1983

Szilvássy Z, Fernandy P, Bor P, Jakab I, Lonovics J, Koltai M (1994) Ventricular overdrive pacing-induced anti-ischemic effect: a conscious rabbit model of preconditioning. Am J Physiol 266(Heart Circ Physiol 35): H2033–H2041

Szilvássy Z, Fernandy P, Szilvássy J, Nagy I, Karcsu S, Lonovics J, Dux L, Koltai M (1995) The loss of pacing-induced preconditioning in atherosclerotic rabbits: role of hypercholesterolaemia. J Mol Cell Cardiol 27:2559–2569

Tamura Y, Chi L, Driscoll EM, Hoff PT, Freeman BA, Gallagher KP, Lucchesi BR (1988) Superoxide dismutase conjugated to polyethylene glycol provides sustained protection against myocardial ischemia/reperfusion injury in canine heart. Circ Res 63:944–959

Toombs CF, McGee DS, Johnston WE, Vinten-Johansen J (1993) Protection from ischaemic-reperfusion injury with adenosine pretreatment is reversed by inhibition of ATP sensitive potassium channels. Cardiovasc Res 27:623–629

van Gilst WH, de Graeff PA, Wesseling H, de Langen CDJ (1986) Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors: a comparison of captopril, enalapril, and HOE 498. J Cardiovasc Pharmacol 8:722–728

Vegh A, Szekeres L, Parrat JR (1990) Protective effects of preconditioning of the ischaemic myocardium involve cyclooxygenase products. Cardiovasc Res 24:1020–1023

Volovsek A, Subramanian R, Reboussin D (1992) Effects of duration of ischaemia during preconditioning on mechanical function, enzyme release and energy production in the isolated working rat heart. J Mol Cell Cardiol 24:1011–1019

Wiemer G, Schölkens BA, Becker RHA, Busse R (1991) Ramiprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium-derived bradykinin. Hypertension 18:558–563

Yang XM, Baxter GF, Heads RJ, Yellon DM, Downey JM, Cohen MV (1996) Infarct limitation of the second window of protection in a conscious rabbit model. Cardiovasc Res 31:777–783

Yao Z, Gross GJ (1994) A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course, and role of K_{ATP} channels. Circulation 89:1229–1236

MRI Studies of Cardiac Function

Purpose and Rationale

Magnetic resonance imaging (MRI) is the preferred technique for the visualization of lesions in the brain and spinal cord of patients with MS. It visualizes the resonance signals of tissue protons when they are placed in a time-varying strong magnetic field. The most frequently used parameters measured in MS are the spin–lattice relaxation time (T_1) and the spin–spin relaxation time (T_2). MRI is routinely used as a tomographic imaging technique, where anatomical pictures are created of 1 mm-thick tissue sections. The contrast differences between brain structures in most MRI techniques are determined by the different densities and diffusion of protons, as well as differences in relaxation times. T_2 images are sensitive to water, and because all pathological alterations in MS brains are associated with altered distribution of tissue water (edema), this technique is highly useful for visualization of the spatial distribution of lesions. Contrast in T_1 images is determined mainly by different lattice densities. Dense structures, such as compact white matter, have low T_1 values, whereas relatively loose structures, such as gray matter or lesions, have higher T_1 values.

To distinguish inflammatory active from inactive lesions, the paramagnetic dye gadolinium-DTPA is intravenously injected (0.1–0.3 mmol kg^{-1}) and, in areas of increased blood–brain barrier permeability, leaks into the brain parenchyma, causing local enhancement of the T_1-weighted signal intensity.

A third important MRI technique in MS is magnetization transfer ratio (MTR) imaging. The MTR of a given tissue is defined as the ratio of free protons versus protons bound to tissue macromolecules.

MRI has emerged as a highly accurate and quantitative tool for the evaluation of cardiac function (Peshock et al. 1996).

Al-Shafei et al. (2002a, b) performed MRI analysis of cardiac cycle events in diabetic rats and tested the effect of angiotensin-converting enzyme inhibition.

Procedure

Diabetes was induced in Wistar rats at an age of 7, 10, and 13 weeks. The rats were anesthetized using 1–2 % halothane in oxygen, and their blood glucose levels were checked. They were then given a single intraperitoneal injection of streptozotocin 65 mg/kg body weight. The control rats received sham injections of the citrated buffer when they were 7 weeks old. One diabetic group was treated with 2 g/l captopril in the drinking water.

For **MR imaging**, rats were anesthetized using 1–2 % halothane in oxygen, weighed, and their systolic blood pressures measured noninvasively using a rat tail blood pressure monitor both before and after imaging sessions to confirm physiological stability. Electrocardiographic (ECG) monitoring used shielded subcutaneous electrodes and a Tektronix 2225 oscilloscope. The cine imaging protocols were performed with the anesthetized animal placed in a specially designed home-built half-sine-spaced birdcage radiofrequency (RF) probe unit contained within a cylindrical plastic holder fitted within a gradient set of internal diameter 11 cm. The RF probe unit was made up of a half-sine-spaced birdcage RF probe of internal diameter of 4.5 cm with open ends, an RF shield consisting of a cylinder of copper gauze surrounding and sliding over the birdcage, a tuning capacitor, and a coaxial cable to carry the RF (Ballon et al. 1990). The assembly included ECG leads, attachment plugs for the ECG leads, and a unit to anchor anesthetic delivery tubes near the nose of the animal. All experiments used a 2T Oxford Instrument (UK) superconducting magnet with a horizontal internal bore of 31 cm. A gated cine protocol synchronized line acquisition to set times following alternate electrocardiographicR waves. This acquisition was then repeated at the same slice position at 12 equally incremented times through the cardiac cycle. This sequence in turn was repeated for each of the 128 lines to generate each 128 × 128 image, which itself was acquired twice for signal averaging. The preceding procedure was in turn repeated 12 times to obtain signal-averaged images for every one of the 12 contiguous transverse slices examined. Each imaging session therefore required (128 × 12 × 2 × 2) times the cardiac cycle duration. The effective repeat time (TR) was approximately 13 ms. The short echo time (TE) of 4.3 ms reduced motion artifacts and ensured good contrast between blood and myocardium.

Evaluation

The image data were transferred from the MRI console using in-house hardware and software to remote UNIX workstations for quantitative analysis using in-house software based on CaMReS libraries (CaMReS, Dr N. J. Herrod, Herchel Smith Laboratory for Medicinal Chemistry, University of Cambridge).

Modifications of the Method

Itter et al. (2004) used noninvasive MRI techniques in a model of chronic heart failure in spontaneously hypertensive rats.

Bryant et al. (1998) and Franco et al. (1999) described MRI and invasive evaluation of development of heart failure in transgenic **mice** with myocardial expression of tumor necrosis factor-α.

Wiesmann et al. (2002) reported analysis of right ventricular function in healthy mice and a murine model of heart failure by in vivo MRI.

Kraitchman et al. (2003) described quantitative ischemia detection during cardiac magnetic resonance stress testing by use of fast harmonic phase MTI (FastHARP) in dogs.

Reddy et al. (2004) discussed the feasibility of a porcine model of healed myocardial infarction by integration of cardiac MRI with three-

dimensional electroanatomic mapping to guide left ventricular catheter manipulation.

Pelzer et al. (2005) reported that the estrogen receptor-α agonist 16α-LE2 inhibits cardiac hypertrophy and improves hemodynamic function in estrogen-deficient spontaneously hypertensive rats. Improved left ventricular function upon 16α-LE2 treatment was also observed in cardiac MRI studies.

References and Further Reading

Al-Shafei AIM, Wise RG, Gresham GA, Bronns G, Carpenter TA, Hall LD, Huang CHL (2002a) Non-invasive magnetic resonance assessment of myocardial changes and the effects of angiotensin-converting enzyme inhibition in diabetic rats. J Physiol (Lond) 538:541–553

Al-Shafei AIM, Wise RG, Gresham GA, Carpenter TA, Hall LD, Huang CHL (2002b) Magnetic resonance imaging analysis of cardiac cycle events in diabetic rats: the effect of angiotensin-converting enzyme inhibition. J Physiol (Lond) 538:555–572

Ballon D, Graham MC, Midownik S, Koutcher JA (1990) A 64 MHz half-birdcage resonator for clinical imaging. J Magn Reson 90:131–140

Bryant D, Becker L, Richardson J, Shelton J, Franco F, Peshock R, Thompson M, Giroir B (1998) Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor-α. Circulation 97:1375–1381

Franco F, Thomas GD, Giroir B, Bryant D, Bullock MC, Chwialkowski MC, Victor RG, Peshock RM (1999) Magnetic resonance imaging and invasive evaluation of development of heart failure in transgenic mice with myocardial expression of tumor necrosis factor-α. Circulation 99:448–454

Itter G, Jung W, Juretschke P, Schölkens BA, Linz W (2004) A model of chronic heart failure in spontaneous hypertensive rats (SHR). Lab Anim 38:138–148

Kraitchman DL, Sampath S, Castillo E, Derbyshire JA, Boston RC, Bluemke DA, Gerber BL, Pronce JL, Osman NF (2003) Quantitative ischemia detection during cardiac magnetic resonance stress testing by use of FastHARP. Circulation 107:2025–2030

Pelzer T, Jazbutyte V, Hu K, Segerer S, Nahrendorf M, Nordbeck P, Bonz AW, Muck J, Fritzemeier KH, Hegele-Hartung C, Ertl G, Neyses L (2005) The estrogen receptor-α agonist 16α-LE2 inhibits cardiac hypertrophy and improves hemodynamic function in estrogen-deficient spontaneously hypertensive rats. Cardiovasc Res 67:604–612

Peshock RM, Willet DL, Sayad DE, Hundley WG, Chwialkowski MC, Clarke GD, Parkey RW (1996) Quantitative MR imaging of the heart. Magn Reson Imaging Clin N Am 4:267–305

Reddy VY, Malchano ZJ, Holmvang G, Schmidt EJ, d'Avila A, Houghtaling C, Chan RC (2004) Integration of cardiac magnetic resonance imaging with three-dimensional electroanatomic mapping to guide left ventricular catheter manipulation. Feasibility of a porcine model of healed myocardial infarction. J Am Coll Cardiol 44:2202–2213

Wiesmann F, Frydrychowicz A, Rautenberg J, Illinger R, Rommel E, Haase A, Neubauer S (2002) Analysis of right ventricular function in healthy mice and a murine model of heart failure by in vivo MRI. Am J Physiol 283: H1065–H1071

MRI Studies After Heart and Lung Transplantation

Purpose and Rationale

Acute cardiac allograft rejection continues to be the cause of graft loss and contributes to the morbidity and mortality after cardiac transplantation. Endomyocardial biopsy is used routinely for cardiac transplant rejection surveillance. A sensitive and noninvasive method for detecting rejection is desirable. Kanno et al. (2001) developed a rat model of heterotopic heart and lung transplantation for MRI experiments. Allotransplantations were performed with syngeneic transplantations serving as controls. MR images were obtained with a gradient echo method.

Procedure

Animals

All rats used in the experiments were male, 2–3 months of age, and weighed 220–250 g each. Animals were housed individually and provided with food and water ad libitum. Inbred Brown Norway (BN; $RT1^n$) and DA ($RT1^a$) rats were obtained from Harlan Sprague Dawley (Indianapolis, Ind).

Heart and Lung Transplantation

Under anesthesia with injection of 35 mg/kg body weight of sodium pentobarbital IP, 500 U/kg body weight of heparin was injected. In the syngeneic group, an en bloc donor heart and lung were taken from a BN rat and transplanted to another BN rat. In the allogeneic group, a graft from a DA rat was transplanted to a BN rat. This group was divided into two groups: one group was treated with 3 mg/kg per day cyclosporine (CsA), and the other group was not given CsA. Graft survival was monitored every day by palpating contraction of the transplanted heart.

Operative procedures have been described by Kanno et al. (2000). In brief, after the chest wall of the donor rat was opened, the left lung was ligated and excised. The azygos vein with the left superior and right superior venae cavae was ligated and divided. The descending thoracic aorta was transected, and 10 ml of cold University of Wisconsin solution (UW solution, Dupont Pharma) was infused into the inferior vena cava until the fluid draining from the aorta was clear, followed by ligation and division of the inferior vena cava. The ascending aorta was dissected and transected at the portion between the left common carotid artery and the left subclavian artery, followed by ligation and division of the right brachiocephalic artery and the left common carotid artery. After removal of the heart and lung from the donor, the right lung was washed three times through the bronchus with UW solution containing penicillin G. The grafts were then placed into cold UW solution for ≈5 min until transplantation. Next, the left inguinal portion of the recipient rat was opened and dissected to make enough space for the transplanted organs. The left lower part of the abdominal wall was opened in a transverse fashion from the left femoral vessels to the midline. The abdominal organs were retracted to the right, and both the aorta and the inferior vena cava just beyond the bifurcation were dissected. The vessels were clamped, and an appropriate opening of the aorta was made to receive the aorta of the graft in an end-to-side fashion. Rhythmic heartbeats commenced spontaneously as the heart and the lung regained circulation after removal of the clamp. After hemostasis of the surgical field, the abdominal wall was sutured, with care taken not to kink or obstruct the aorta of the graft.

MRI Experiments

MRI measurements were carried out on a 4.7-T/40 cm Bruker AVANCE DRX MR instrument equipped with 15 cm, 10 gauss/cm shielded gradients. In vivo MR images of transplanted heart–lung were obtained over a period of 24 h after infusion of dextran-coated ultrasmall superparamagnetic iron oxide (USPIO) particles. The imaging sequence consisted of a gradient echo sequence, triggered to ECG and ventilator (60 strokes/min, 10 ml/kg), with TR/TE 500/10 ms, flip angle equal to Ernst angle, slice thickness 1 mm, field of view 6.0 cm, data matrix size 256 × 130 (zero filled to 256 × 256), and scan time 5 min. ECG leads were placed on both of the hind limbs of the rat with the transplant to pick up the heartbeat from the transplanted heart more effectively. The change of MRI signal intensity was measured in whole ventricular wall in each transplanted heart. The MR signal intensity of the heart was normalized to that of the leg muscle, because USPIO particles are not readily taken up by muscular tissue, according to Gellissen et al. (1999).

Dextran-coated USPIO particles were synthesized according to the method of Palmacci and Josephson (1993) with slight modifications (Dodd et al. 1999). The MR relaxivities R_1 (spin–lattice relaxation rate constant, $1/T_2$, per mol of Fe in USPIO) and R_2 (spin–spin relaxation rate constant, $1/T_2$, per mol of Fe in USPIO) measured at 4.7 T were 3.8×10^4 and 9.1×10^4 (mol/l)/s, respectively. For in vivo studies, dextran-coated USPIO particles were dialyzed against PBS solution and diluted to a concentration of 18 μmol Fe/ml, and 0.8 ml of the suspension (i.e., ≈3 mg Fe/kg body weight) was injected intravenously for each study.

At 6 days after transplantation, dextran-coated USPIO particles were injected intravenously as mentioned above, and the animals were subjected to MRI. Then, 24 h later, these animals were again placed inside the magnet and scanned. The regions of interest were defined manually with Bruker software. MR signal intensity in the entire ventricular wall in the plane was measured. After injection of USPIO particles at postoperative day (POD) 6, animals with allotransplants were given CsA for 4 (POD 7–10) or 7 (POD 7–13) days and reinjected with USPIO particles on POD 14.

Pathological Analysis and Immunohistochemistry

After an MR experiment was completed, the transplanted hearts were extirpated, fixed in 3.7 % formaldehyde, and embedded in paraffin for 5 5 µm mu;m sections. Hematoxylin–eosin staining and Perl's Prussian blue staining were performed in the Transplantation Pathology Laboratory of the University of Pittsburgh Medical Center. Histological analysis for pathological grading of heart rejection, which is based on the criteria established by the International Society for Heart and Lung Transplantation, was also performed by this laboratory in a blinded manner. Monoclonal antirat macrophage antibody (ED1, Serotec) was used as a primary antibody for macrophages. Immunohistochemistry was carried out with the ABC staining system (Santa Cruz Biotechnology) according to the manufacturer's protocol.

Evaluation

The results are presented as mean ± SD. The results were analyzed by ANOVA with StatView software (SAS Institute). A value of $P < 0.05$ was considered to be statistically significant.

References and Further Reading

Dodd SJ, Williams M, Suhan JP et al (1999) Detection of single mammalian cells by high-resolution magnetic resonance imaging. Biophys J 76:103–109

Gellissen J, Axmann C, Prescher A et al (1999) Extra- and intracellular accumulation of ultrasmall superparamagnetic iron oxides (USPIO) in experimentally induced abscesses of the peripheral soft tissues and their effects on magnetic resonance imaging. Magn Reson Imaging 17:557–567

Kanno S, Lee PC, Dodd SJ et al (2000) A novel approach using magnetic resonance imaging for the detection of lung allograft rejection. J Thorac Cardiovasc Surg 120:923–934

Kanno S, Wu YJ, Lee PC, Dodd SJ, Williams M, Griffith HP, Ho C (2001) Macrophage accumulation associated with rat cardiac allograft rejection detected by magnetic resonance imaging with ultrasmall superparamagnetic iron oxide particles. Circulation 104:934–938

Palmacci S, Josephson L (1993) Synthesis of polysaccharide covered superparamagnetic oxide colloids. US Patent 5,262,176 Example 1, 16 Nov 1993

Ex Vivo Methods

Plastic Casts from Coronary Vasculature Bed

Purpose and Rationale

Prolonged administration of coronary drugs has been shown to increase the number and size of interarterial collaterals of dogs and pigs after coronary occlusion (Vineberg et al. 1962; Meesmann and Bachmann 1966). An increased rate of development of collateral arteries was observed after physical exercise in dogs (Schaper et al. 1965), as well as after chronic administration of coronary dilating drugs (Lumb and Hardy 1963). An even more effective stimulus for collateral development is an acute or gradual occlusion of one or several major coronary branches. Filling the arterial coronary bed with a plastic provides the possibility to make the collaterals visible and to quantify them (Schmidt and Schmier 1966; Kadatz 1969).

Procedure

Dogs weighing 10–15 kg are anesthetized with pentobarbital sodium 30 mg/kg i.v. They are respirated artificially and the thorax is opened. After opening of the pericard, ameroid cuffs are placed around major coronary branches. Gradual

swelling of the plastic material occludes the lumen within 3–4 weeks. The dogs are treated daily with the test drug or placebo. After 1 week recovery period, they are submitted to exercise on a treadmill ergometer. After 6 weeks treatment, the animals are sacrificed, the heart removed, and the coronary bed flushed with saline. The liquid plastic Araldite is used to fill the whole coronary tree from the bulbus aortae. The aortic valves are glued together in order to prevent filling of the left ventricle. Red colored Araldite is used to fill the arterial tree. The venous part of the coronary vasculature can be filled with blue colored Araldite from the venous sinus. The uniformity of the filling pressure, the filling time, and the viscosity of the material are important. Polymerization is complete after several hours. Then, the tissue is digested with 35 % potassium hydroxide. The method gives stable preparations which can be preserved for a long time.

Evaluation

Plastic casts from drug-treated animals are compared with casts from dogs submitted to the same procedure without drug treatment.

Critical Assessment of the Method

The procedure allows impressive demonstration of the formation of arterial collaterals. The results of postmortem Araldite impletion agree with the functional results of experimental coronary occlusion.

Modifications of the Method

Boor and Reynolds (1977) described a simple planimetric method for the determination of left ventricular mass and necrotic myocardial mass in postmortem hearts.

References and Further Reading

Boor PJ, Reynolds ES (1977) A simple planimetric method for determination of left ventricular mass and necrotic myocardial mass in postmortem hearts. Am J Clin Pathol 68:387–392

Kadatz R (1969) Sauerstoffdruck und Durchblutung im gesunden und koronarinsuffizienten Myocard des Hundes und ihre Beeinflussung durch koronarerweiternde Pharmaka. Arch Kreislaufforsch 58:263–293

Kadatz R (1971) Agents acting on coronary blood vessels. In: Turner RA, Hebborn P (eds) Screening methods in pharmacology, vol II. Academic Press, New York/London, pp 41–60

Lumb G, Hardy LB. (1963) Collateral circulation in the heart. N C Med J. 24:456–60.

Meesman W (1982) Early arrhythmias and primary ventricular fibrillation after acute myocardial ischemia in relation to pre-existing collaterals. In: Parratt JR (ed) Early arrhythmias resulting from myocardial ischemia. Mechanisms and prevention by drugs. McMillan, London, pp 93–112

Meesmann W, Bachmann GW (1966) Pharmakodynamisch induzierte Entwicklung von Koronar-Kollateralen in Abhängigkeit von der Dosis. Arzneim Forsch 16:501–509

Meesmann W, Schulz FW, Schley G, Adolphsen P (1970) Überlebensquote nach akutem experimentellem Coronarverschluß in Abhängigkeit von Spontankollateralen des Herzens. Z Ges Exp Med 153:246–264

Schaper W, Xhonneux R, Jageneau AHM (1965) Stimulation of the coronary collateral circulation by Lidoflazine (R 7904). Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 252:1–8

Schmidt HD, Schmier J (1966) Eine Methode zur Herstellung anatomischer Korrosionspräparate – dargestellt am Koronargefäßsystem des Hundes. Zschr Kreislaufforsch 55:297–305

Vineberg AM, Chari RS, Pifarré R, Mercier C (1962) The effect of Persantin on intercoronary collateral circulation and survival during gradual experimental coronary occlusion. Can Med Assoc J 87:336–345

References and Further Reading

Heart–Lung Preparation

Beaconsfield P, Oakley C, Carpi A, Rainsbury R, del Basso P (1974) Cardiac effect of delta-9-tetrahydrocannabinol on a heart-lung preparation and on the intact animal. Eur J Cardiol 2:167–173

Beaufort AM, Wierda JMKH, Houwertjes MC, Kleef UW, Meijer DKF (1993) The isolated heart-lung preparation

in the cat. An *in situ* model to study the role of the lungs in the disposition of drugs. J Pharmacol Toxicol Methods 29:147–156

Caffrey JL, Wooldridge CB, Gaugl JF (1986) Naloxone enhances myocardial responses to isoproterenol in dog isolated heart-lung. Am J Physiol 250(Heart Circ Physiol 19):H749–H754

Capri A, Oliverio A (1965) Effect of reserpine on the heart-lung preparation of guinea pig. Arch Int Pharmacodyn Ther 157:470–486

Dietz JR (1984) Release of natriuretic factor from rat heart-lung preparation by atrial distension. Am J Physiol 247 (Regulatory Integrative Comp Physiol 16): R1093–R1096

Dietz JR (1987) Control of atrial natriuretic factor release from a rat heart-lung preparation. Am J Physiol 252 (Regulatory Integrative Comp Physiol 21):R498–R502

Fessler HE, Brower RG, Wise RA, Permutt S (1988) Mechanism of reduced LV afterload by systolic and diastolic positive pleural pressure. J Appl Physiol 65:1244–1250

Fukuse T, Albes JM, Takahashi Y, Brandes H, Hausen B, Schäfers HJ (1995) Influence of red blood cells in an ex vivo rat heart-lung model. J Surg Res 59:399–404

Hausknecht MJ, Wise RA, Brower RG, Hassapoyannes C, Weisfeldt ML, Suzuki J, Permutt S (1986) Effects of lung inflation on blood flow during cardiopulmonary resuscitation in the canine isolated heart-lung preparation. Circ Res 59:676–683

Iizuka H (1983) Cardiac effects of acetylcholine and its congeners as assessed in canine heart-lung preparation. Folia Pharmacol Jpn 81:441–449

Imai S, Shigei T, Hashimoto K (1961) Cardiac actions of methoxamine with special reference to its antagonistic action to epinephrine. Circ Res 9:552–560

Ishikawa N, Taki K, Hojo Y, Hagino Y, Shigei T (1978) Direct recording of cardiac output- and venous return-curves in the dog heart-lung preparation for a graphical analysis of the effects of cardioactive drugs. Jpn Heart J 19:775–782

Ishikawa N, Taki K, Hojo Y, Hagino Y, Shigei T (1983) Graphical analysis of drug effects in the dog heart-lung preparation – with particular reference to the pulmonary circulation and effects of norepinephrine and 5-hydroxytryptamine. Jpn J Pharmacol 33:785–794

Jerusalem E, Starling EH (1910) On the significance of carbon dioxide for the heart beat. J Physiol 40:279–294

Kashimoto S, Tsuji Y, Kumazawa T (1987) Effects of halothane and enflurane on myocardial metabolism during postischaemic reperfusion in the rat. Acta Anaesth Scand 31:44–47

Kashimoto S, Kume M, Kumazawa T (1990) Functional and metabolic effects of bupivacaine and lignocaine in the rat heart-lung preparation. Br J Anaesth 65:521–526

Kashimoto S, Nakamura T, Kume M, Nonaka A, Kumazawa T (1994) Effects and interaction of nicardipine and volatile anesthetics in the rat heart-lung preparation. J Anesth 8:78–83

Kashimoto S, Namakura T, Furuya A, Kume M, Kumazawa T (1995) Alteration of cardiac function and metabolism in the rat heart-lung preparation by methyl methacrylate and their protection by ulinastatin. Jpn J Anesth 44:1477–1481

Kissling G, Blickle B, Pascht U (2000) Modified heart-lung preparation for the evaluation of systolic and diastolic coronary flow in rats. Am J Physiol Heart Circ Physiol 278(1):H277–H284

Knowlton FP, Starling EH (1912) The influence of variations in temperature and blood pressure on the performance of the isolated mammalian heart. J Physiol 44:206–219

Kontos GJ Jr, Borkon AM, Adachi H, Baumgartner WA, Hutchins GM, Brawn J, Reitz BA (1987) Successful extended cardiopulmonary preservation in the autoperfused working heart-lung preparation. Surgery 102:269–276

Kontos GJ Jr, Borkon AM, Baumgartner WA, Fonger JD, Hutchins GM, Adachi H, Galloway E, Reitz BA (1988) Improved myocardial and pulmonary preservation by metabolic substrate enhancement in the autoperfused working heart-lung preparation. J Heart Transplant 7:140–144

Krayer O (1931) Versuche am isolierten Herzen. Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 162:1–28

Krayer O, Mendez R (1942) Studies on veratrum alkaloids. I. The action of veratrine upon the isolated mammalian heart. J Pharmacol Exper Ther 74:350–364

Merin RG (1988) The isolated heart preparation. Br J Anesth 60:28S–34S

Muskett AD, Burton NA, Gay WA, Miller M, Rabkin MS (1986) Preservation in the rabbit autoperfusing heart-lung preparation: a potential role of indomethacin. Surg Forum 37:252–254

Muskett AD, Burton NA, Grossman M, Gay WA Jr (1988) The rabbit autoperfusing heart-lung preparation. J Surg Res 44:104–108

Naka Y, Hirose H, Matsuda H, Nakano S, Shirakura R, Kawaguchi A, Miyamoto Y, Miyagawa S, Fukushima N, Kawashima Y (1989) Prevention of pulmonary edema in autoperfusing heart-lung preparations by FUT-175 and leukocyte depletion. Transplant Proc 21:1353–1356

Namakura J, Zhang S, Ishikawa N (1987) Role of pulmonary innervation in canine *in situ* lung-perfusion preparation: a new model of neurogenic pulmonary edema. Clin Exp Pharmacol Physiol 14:535–543

Ono H, O'Hara N (1984) A study of the cardiodepressant action of a β-blocking agent carteolol in heart-lung preparation of the dog. Jpn Circ J 86:1030–1044

Ono H, Kanazawa Y, O'Hara N, Hashimoto K (1984) Estimation of cardiodepressant potency of nadolol, alprenololol, propranolol and pindolol, β-blocking agents, in heart-lung preparation and blood-perfused excised papillary muscle preparation of the dog. Jpn J Pharmacol 36:507–517

Onwochei MO, Rapp JP (1988) Biochemically stimulated release of atrial natriuretic factor from heart-lung preparation in Dahl rats. Proc Soc Exp Biol Med 188:395–404

Onwochei MO, Snajdar RM, Rapp JP (1987) Release of atrial natriuretic factor from heart-lung preparations of inbred Dahl rats. Am J Physiol 253(Heart Circ Physiol 22):H1044–H1052

Riveron FA, Ross JH, Schwartz KA, Casey G, Sanders ON, Eisiminger R, Magilligan DJ Jr (1988) Energy expenditure of autoperfusing heart-lung preparation. Circulation 78:II Suppl III-103–III-109

Robicsek F, Masters TN, Duncan GD, Denyer MH, Rise HE, Etchison M (1985) An autoperfused heart-lung preparation: metabolism and function. Heart Transplant 4:334–338

Seifen E, Seifen AB, Kennedy RH, Bushman GA, Loss GE, Williams TG (1987) Comparison of cardiac effects of enflurane, isoflurane, and halothane in the dog heart-lung preparation. J Cardiothorac Anesth 1:543–553

Seifen E, Kennedy RH, Seifen AB (1988) Interaction of BAY K-8644 with effects of digoxin in the dog heart-lung preparation. Eur J Pharmacol 158:109–117

Shigei T, Hashimoto K (1960) Study on the mechanism of the heart failure induced by pentobarbital, quinine, fluoroacetate and dinitrophenol in dog's heart-lung preparation and effects of sympathomimetic amines and ouabain on it. Jpn J Pharmacol 9:109–122

Somani P, Blum BK (1966) Blockade of epinephrine- and ouabain-induced cardiac arrhythmias in the dog heart-lung preparation. J Pharmacol Exp Ther 152:235–242

Takeda K, Iizuka K, Imai S (1973) Cardiac actions of oxprenolol as studied in dog heart-lung preparations. Arzneim Forsch/Drug Res 23:1446–1450

Wollenberger A (1947) On the energy-rich phosphate supply of the failing heart. Am J Physiol 150:733–745

Isolated Heart According to Langendorff

Avkiran M, Curtis MJ (1991) Independent dual perfusion of left and right coronary arteries in isolated rat hearts. Am J Physiol 261(Heart Circ Physiol 30): H2082–H2090

Balderston SM, Johnson KE, Reiter MJ (1991) Electrophysiologic evaluation of cardiovascular agents in the isolated intact rabbit heart. J Pharmacol Methods 25:205–213

Bardenheuer H, Schrader J (1983) Relationship between myocardial oxygen consumption, coronary flow, and adenosine release in an improved isolated working heart preparation of guinea pigs. Circ Res 51:263–271

Barr RL, Lopaschuk GD (1997) Direct measurement of energy metabolism in the isolated rat heart. J Pharmacol Toxicol Methods 38:11–17

Bittner HB, Chen EP, Peterseim DS, Van Trigt P (1996) A work-performing heart preparation for myocardial performance analysis in murine hearts. J Surg Res 64:57–62

Bratkovsky S, Aasum E, Birkeland CH, Riemersma RA, Myrhe ESP, Larsen TS (2004) Measurement of coronary flow reserve in isolated hearts from mice. Acta Physiol Scand 181:167–172

Broadley KJ (1979) The Langendorff heart preparation – reappraisal of its role as a research and teaching model for coronary vasoactive drugs. J Pharmacol Methods 2:143–156

Brooks WW, Apstein CS (1996) Effect of treppe on isovolumic function in the isolated blood-perfused mouse heart. J Mol Cell Cardiol 28:1817–1822

Brown TG, Lands AM (1964) Cardiovascular activity of sympathomimetic amines. In: Laurence DR, Bacharach AL (eds) Evaluation of drug activities: pharmacometrics. Academic Press, London/New York, pp 353–368

Burn HJ, Goodford PJ (1957) Effect of lack of glucose and of lack of oxygen on ventricular fibrillation. J Physiol 137:20P–21P

Burn HJ, Hukovic S (1960) Anoxia and ventricular fibrillation: with a summary of evidence on the cause of fibrillation. Br J Pharmacol 15:67–70

Chevalier B, Mouas C, Mansier P, Aumont MC, Swynghedauw B (1987) Screening of inotropic drugs on isolated rat and guinea pig hearts. J Pharmacol Methods 17:313–326

Dhein S, Müller A, Klaus W (1989) The potential of epicardial activation mapping in isolated hearts for the assessment of arrhythmogenic and antiarrhythmic drug activity. J Pharmacol Methods 22:197–206

Döring HJ (1990) The isolated perfused warm-blooded heart according to LANGENDORFF. Technique – function – application. Physiologie Bohemoslov 39:481–496

Flynn SB, Gristwood RW, Owen DAA (1978) Characterization of an isolated, working heart guinea-pig heart including effects of histamine and noradrenaline. J Pharmacol Methods 1:183–195

Garlick PP, Radda GK, Seeley PJ, Chance B (1977) Phosphorus NMR studies on perfused heart. Biochem Biophys Res Commun 74:1256–1262

Gottlieb R, Magnus R (1904) Digitalis und Herzarbeit. Nach Versuchen an überlebenden Warmblüterherzen. Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 51:30–63

Hannan RL, John MC, Kouretas PC, Hack BD, Matherne GP, Laubach VE (2000) Deletion of endothelial nitric oxidase exacerbates myocardial stunning in an isolated mouse heart model. J Surg Res 93:127–132

Hendrikx M, Mubagawa K, Verdonk F, Overloop K, Van Hecke P, Vanstapel F, Van Lommel A, Verbeken E, Lauweryns J, Flameng W (1994) Na^+-H^+ exchange inhibitor HOE 694 improves postischemic function and high-energy phosphate resynthesis and reduces

Ca^{2+} overload in isolated perfused rabbit heart. Circulation 89:2787–2798

Hollis DP, Nunnally RL, Taylor GJ, Weisfeldt ML, Jacobus WE (1978) Phosphorus NMR studies of heart physiology. J Magn Reson 29:319–330

Hukovic S, Muscholl E (1962) Die Noradrenalin-Abgabe aus dem isolierten Kaninchenherzen bei sympathischer Nervenreizung und ihre pharmakologische Beeinflussung. Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 244:81–96

Igic R (1996) The isolated perfused "working" rat heart: a new method. J Pharmacol Toxicol Methods 35:63–67

Ishiu R, Abe Y, Onishi K, Ueda Y, Sekioka K, Nakano T (1996) Changes in calcium transient and left ventricular function during inotropic stimulation and myocardial ischemia in indo-1-loaded beating guinea pig heart. J Pharmacol Toxicol Methods 35:55–61

Jacobus WE, Taylor GJ, Hollins DP, Nunnally RL (1977) Phosphorus nuclear magnetic resonance of perfused working rat hearts. Nature 265:756–758

Krzeminski T, Kurcok A, Kapustecki J, Kowalinski J, Slowinski Z, Brus R (1991) A new concept of the isolated heart preparation with on-line computerized data evaluation. J Pharmacol Methods 25:95–110

Lamontagne D, König A, Bassenge E, Busse R (1992) Prostacyclin and nitric oxide contribute to the vasodilator action of acetylcholine and bradykinin in the intact rabbit coronary bed. J Cardiovasc Pharmacol 20:652.657

Langendorff O (1895) Untersuchungen am lebenden Säugethierherzen. Pflüger's Arch Ges Physiol 61:291–332

Lindner E (1963) Untersuchungen über die flimmerwidrige Wirkung des *N*-(3′-phenylpropyl-(2′))-1,1-diphenylpropyl-(3)-amins (Segontin). Arch Int Pharmacodyn 146:485–500

Lindner E, Grötsch H (1973) Methode zur graduellen Bestimmung hypoxischer Schädigung am isolierten Meerschweinchenherzen nach Langendorff. Arzneim Forsch/Drug Res 23:926–929

Lindner E, Hajdu P (1968) Die fortlaufende Messung des Kaliumverlustes des isolierten Herzens zur Bestimmung der Wirkungsstärke digitalisartiger Körper. Arch Int Pharmacodyn 175:365–372

Linz W, Schölkens BA, Han YF (1986) Beneficial effects of the converting enzyme inhibitor, ramipril, in ischemic rat hearts. J Cardiovasc Pharmacol 8(Suppl 10): S91–S99

Linz W, Martorana PA, Schölkens BA (1990) Local inhibition of bradykinin degradation in ischemic hearts. J Cardiovasc Pharmacol 15(Suppl 6):S99–S109

Matthews PM, Radda GK (1984) Applications of nuclear magnetic resonance to the study of myocardial metabolism and pharmacology. In: Schwartz A (ed) Methods in pharmacology. Myocardial biology, vol 5. Plenum Press, New York/London, pp 175–228

Michio F, Hideo I, Tetsuya A (1985) *In vitro* assessment of myocardial function using a working rabbit heart. J Pharmacol Methods 14:49–60

Mouren S, Vicaut E, Lamhaut L, Riou B, Ouattara A (2010) Crystalloid versus red blood cell-containing medium in the Langendorff-perfused isolated heart preparation. Eur J Anaesthesiol 27(9):780–787

Neely JR, Liebermeister H, Batterbsy EJ, Morgan HE (1967) Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol 212:804–814

Plumier JCL, Ross BM, Currie RW, Angelidis CA, Kazlaris H, Kollias G, Pagoulatos GN (1995) Transgenic mice expressing the human heart heat shock protein 70 have improved postischemic myocardial recovery. J Clin Invest 95:1854–1860

Ross BD (1972) Chapter 5: Heart and skeletal muscle. In: Perfusion techniques in biochemistry. A laboratory manual in the use of isolated perfused organs in biochemical experimentation. Clarendon Press, Oxford, pp 258–320

Ross SA, Rorabaugh BR, Chalothorn D, Yun J, Gonzalez-Cabrera PJ, McCune DF, Piasik MT, Perez DM (2003) The α_{1B}-adrenergic receptor decreases the inotropic response in the mouse Langendorff heart model. Cardiovasc Res 60:598–607

Rothaul AL, Broadley KJ (1982) Measurements of oxygen tension in perfusates from guinea pig isolated hearts and the demonstration of coronary vasodilator material. J Pharmacol Methods 7:91–103

Sakai K, Akima M, Tsuyama K (1983) Evaluation of the isolated perfused heart of mice, with special reference to vasoconstriction caused by intracoronary acetylcholine. J Pharmacol Methods 10:263–270

Sheikh F, Sontag DP, Fandrich RR, Kardami E, Cattini PA (2001) Overexpression of FGF-2 increases cardiac myocyte viability after injury in isolated mouse hearts. Am J Physiol Heart Circ Physiol 280:H1030–H1050

Sumaray MS, Yellon DM (1998a) Characterization and validation of a murine model of global ischaemia-reperfusion injury. Mol Cell Biochem 186:61–68

Sumaray MS, Yellon DM (1998b) Ischemic preconditioning reduces infarct size following global ischemia in the murine myocardium. Basic Res Cardiol 93:384–390

Sutherland FJ, Shattock MJ, Baker KE, Hearse DJ (2003) Mouse isolated perfused heart: characteristics and cautions. Clin Exp Pharmacol Physiol 30:867–898

Takeo S, Tanonaka K, Liu JX, Ohtsuka Y (1992) Protective effects of antiarrhythmic agents on oxygen-deficiency-induced contractile dysfunction of isolated perfused hearts. In: Yasuda H, Kawaguchi H (eds) New aspects in the treatment of failing heart. Springer, Tokyo/Berlin/Heidelberg, pp 13–219

Tejero-Taldo MI, Gursoy E, Zhao TC, Kukreja RC (2002) α-Adrenergic receptor stimulation produces late preconditioning through inducible nitric oxide synthase in mouse heart. J Mol Cell Cardiol 34:185–195

Wang QD, Swärdh A, Sjöquist PO (2001) Relationship between ischemic time and ischemia/reperfusion injury in isolated Langendorff-perfused mouse hearts. Act Physiol Scand 171:123–128

Xiang JZ, Linz W, Becker H, Ganten D, Lang RE, Schölkens B, Unger T (1985) Effects of converting enzyme inhibitors: ramipril and enalapril on peptide action and neurotransmission in the isolated heart. Eur J Pharmacol 113:215–223

Zander B, Euler H (1976) Concentration measurements of physically dissolved oxygen by the classical van Slyke principle. In: Deng H, Balslen J, Brook R (eds) Measurement of oxygen. Elsevier Scientific Publ. Co, Amsterdam, pp 271–276

Zegner M, Podesser B, Koci G, Weisser J, Hallström S, Schima H, Wollenek GH (1996) Evaluation of the influences of ramiprilat on the reperfusion – Studied on the isolated working heart model. Acta Chir Austriatica 28:343–346

Coronary Artery Ligation in Isolated Working Rat Heart

Grupp IL, Grupp G (1984) Isolated heart preparations perfused or superfused with balanced salt solutions. In: Schwartz A (ed) Methods in pharmacology. Myocardial biology, vol 5. Plenum Press, New York/London, pp 111–128

Igic R (1996) The isolated perfused "working" rat heart: a new method. J Pharmacol Toxicol Methods 35:63–67

Kannengieser GJ, Lubbe WF, Opie LH (1975) Experimental myocardial infarction with left ventricular failure in the isolated perfused rat heart. Effects of isoproterenol and pacing. J Mol Cell Cardiol 7:135–151

Lee HC, Mohabir R, Smith N, Franz MR, Clusin WT (1988) Effect of ischemia on calcium-dependent fluorescence transients in rabbit hearts containing indo 1. Correlation with monophasic action potentials and contraction. Circulation. 78(4):1047–59.

Linz W, Schölkens BA, Han YF (1986a) Beneficial effects of the converting enzyme inhibitor, ramipril, in ischemic rat hearts. J Cardiovasc Pharmacol 8(Suppl 10):S91–S99

Linz W, Schölkens BA, Manwen J, Wilhelm M, Ganten D (1986b) The heart as a target for converting enzyme inhibitors: studies in ischaemic isolated working hearts. J Hypertens 4(Suppl 6):S477–S479

Linz W, Schölkens BA, Kaiser J, Just M, Bei-Yin Q, Albus U, Petry P (1989) Cardiac arrhythmias are ameliorated by local inhibition of angiotensin formation and bradykinin degradation with the converting-enzyme inhibitor ramipril. Cardiovasc Drugs Ther 3:873–882

Linz W, Wohlfart P, Schoelkens BA, Becker RHA, Malinski T, Wiemer G (1999) Late treatment with ramipril increases survival in old spontaneously hypertensive rats. Hypertension 34:291–295

Martorana PA, Linz W, Göbel H, Petry P, Schölkens BA (1987) Effects of nicainoprol on reperfusion arrhythmia in the isolated working rat heart and on ischemia and reperfusion arrhythmia and myocardial infarct size in the anesthetized rat. Eur J Pharmacol 143:391–401

Pepe S, McLennan PL (1993) A maintained afterload model of ischemia in erythrocyte-perfused isolated working hearts. J Pharmacol Toxicol Methods 29:203–210

Rajagopalan R, Ghate AV, Subbarayan P, Linz W, Schoelkens BA (1993) Cardiotonic activity of the water soluble forskolin derivative 8,13-epoxy-6β-(piperidinoacetoxy)-1α,7β,9α-trihydro-xy-labd-14-en-11-one. Arzneim Forsch/Drug Res 43:313–319

Schölkens BA, Linz W, Lindpaintner K, Ganten D (1987) Angiotensin deteriorates but bradykinin improves cardiac function following ischaemia in isolated rat hearts. J Hypertens 5(Suppl 5):S7–S9

Scholz W, Albus U, Linz W, Martorana P, Lang HJ, Schölkens BA (1992) Effects of Na^+/H^+ exchange inhibitors in cardiac ischaemia. J Mol Cell Cardiol 24:731–740

Scholz W, Albus U, Lang HJ, Linz W, Martorana PA, Englert HC, Schölkens BA (1993) Hoe 694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischemia. Br J Pharmacol 109:562–568

van Gilst WH, de Graeff PA, Wesseling H, de Langen CDJ (1986) Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors: a comparison of captopril, enalapril, and HOE 498. J Cardiovasc Pharmacol 8:722–728

Vleeming W, van der Wouw PA, van Rooij HH, Wemer J, Porsius AJ (1989) *In vitro* method for measurement of cardiac performance and responses to inotropic drugs after experimentally induced myocardial infarction in the rat. J Pharmacol Methods 21:95–102

Vogel WM, Lucchesi BR (1980) An isolated, blood perfused, feline heart preparation for evaluating pharmacological interventions during myocardial ischemia. J Pharmacol Methods 4:291–303

Isolated Working Heart Model in Infarcted Rat Heart

Holm S (1979) A simple sequential rejective multiple test procedure. Scand J Stat 6:65–70

Itter G, Jung W, Juretschke P, Schölkens BA, Linz W (2004a) A model of chronic heart failure in spontaneous hypertensive rats (SHR). Lab Anim 38:138–148

Itter G, Jung W, Schölkens BA, Linz W (2004b) The isolated working heart model in infarcted rat hearts. Lab Anim 39:178–193

Relaxation of Bovine Coronary Artery

Dusting GJ, Moncada S, Vane JR (1977) Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins 13:3–15

Gilmore N, Vane JR, Wyllie JH (1968) Prostaglandins released by the spleen. Nature 218:1135–1140

Li J, Matsuura JE, Waugh DJJ, Adrian TE, Abel PW, Manning MC, Smith DD (1997) Structure – activity studies on position 14 of human α-calcitonin gene-related peptide. J Med Chem 40:3071–3076

Isoproterenol Induced Myocardial Necrosis in Rats

Bhargava AS, Preus M, Khater AR, Günzel P (1990) Effect of iloprost on serum creatine kinase and lactate dehydrogenase isoenzymes after isoprenaline-induced cardiac damage in rats. Arzneim Forsch/Drug Res 40:248–252

Brodowicz GR, Lamb DR (1991) Exercise training, indomethacin, and isoproterenol-induced myocardial necrosis in rats. Basic Res Cardiol 86:40–48

Campell JD, Paul RJ (1993) Effects of diltiazem on force, $[Ca^{2+}]_i$, and energy metabolism in porcine coronary artery. J Cardiovasc Pharmacol 22:408–415

Ciplea AG, Kretschmar R, Heimann W, Kirchengast M, Safer A (1988) Protective effect of the new calcium antagonist Anipamil against isoprenaline-induced cardionecrosis in rats. Arzneim-Forsch/Drug Res 38:215–221

Classen L, Michalsky G, Kammermeier H (1993) Catecholamine-induced cardiac necroses: protective effect of leucocytopenia, influence of an S2 antagonist, thromboxansynthetase inhibitor and prostacyclin analogue. Basic Res Cardiol 88:52–59

Ferrans VJ, Hibbs RG, Black WC, Weilbaecher DG (1964) Isoproterenol-induced myocardial necrosis. A histochemical and electron microscopic study. Am Heart J 68:71–90

Genovese A, Chiariello M, de Alfieri W, Latte S, Ferro G, Condorelli M (1982) Quantitative assessment of infarct size in isoproterenol-infarcted rats. Jpn Heart J 23:997–1006

Handforth CP (1962) Isoproterenol-induced myocardial infarction in animals. Arch Pathol 73:161–165

Joseph X, Bloom S, Pledger G, Balazs T (1983) Determinants of resistance to the cardiotoxicity of isoproterenol in rats. Toxicol Appl Pharmacol 69:199–205

Knufman NMJ, van der Laarse A, Vliegen HW, Brinkman CJJ (1987) Quantification of myocardial necrosis and cardiac hypertrophy in isoproterenol-treated rats. Res Commun Chem Pathol Pharmacol 57:15–32

Meijer AEFH, Hettwer H, Ciplea AG (1988) An enzyme histochemical study of isoproterenol-induced myocardial necroses in rats. Histochem J 20:697–707

Preus M, Bhargava AS, Khater AER, Günzel P (1988) Diagnostic value of serum creatine kinase and lactate dehydrogenase isoenzyme determinations for monitoring early cardiac damage in rats. Toxicol Lett 42:225–253

Rona G (1967) Experimental drug-induced myocardial infarction for animal pharmacologic screening. In: Siegeler PE, Moyer JH (eds) Animal and clinical pharmacologic techniques in drug evaluation, vol II. Year Book Medical, Chicago, pp 464–470

Rona G, Chappel CI, Balazs T, Gaudry R (1959) An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Arch Pathol 76:443–455

Rona G, Chappel CI, Kahn DS (1963) The significance of factors modifying the development of isoproterenol-induced myocardial necrosis. Am Heart J 66:389–395

Vértesi C, Knopf E, Gaál KPS (1991) Comparison of the cardioprotective effects of nitroglycerin, molsidomine, and SIN-1 in rats. J Cardiovasc Pharmacol 17(Suppl 3): S141–S144

Wexler BC (1985) Prolonged protective effects following propranolol withdrawal against isoproterenol-induced myocardial infarction in normotensive and hypertensive rats. Br J Exp Pathol 66:143–154

Yang J, Zhao D, Chang YZ, Tin Q, Zhao YT, Shi XY, Zhang ZK, Tang CS (1996) Protective effect of adrenomedullin$^{10-52}$ on isoproterenol-induced myocardial injury in rats. Chin Pharmacol Bull 12:530–533

Myocardial Infarction After Coronary Ligation in Rodents

Azizi M, Rousseau A, Ezan E, Guyene TT, Michelet S, Grognet JM, Lefant M, Corvol P, Ménard J (1996) Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator *N*-acetylseryl-aspartyl-lysyl-proline. J Clin Invest 97:839–844

Azizi M, Ezan E, Nicolet L, Grognet JM, Ménard J (1997a) High plasma level of *N*-acetyl-seryl-aspartyl-lysyl-proline. A new marker of chronic angiotensin-converting enzyme inhibition. Hypertension 30:1015–1019

Azizi M, Rousseau A, Ezan E, Guyene TT, Michelet S, Grognet JM, Lenfant M, Corvol P, Menard J (1997b) Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator *N*-acetylseryl-aspartyl-lysyl-proline. J Clin Invest 97:839–844

Bäcklund T, Palojoki E, Saraste A, Eriksson A, Finkenberg P, Kytö V, Lakkisto P, Mervaala E, Voipio-Pulkki LM, Laine M, Tikkanen L (2004) Sustained cardiomyocyte apoptosis and left ventricular remodelling after myocardial infarction in experimental diabetes. Diabetologia 47:325–330

Belichard P, Savard P, Cardinal R, Naddeau R, Gosselin H, Paradis P, Rouleau JL (1994) Markedly different effects on ventricular remodelling result in a decrease in inducibility of ventricular arrhythmias. J Am Coll Cardiol 23:505–513

Bernauer W (1985) The effect of β-adrenoceptor blocking agents on evolving myocardial necrosis in coronary ligated rats with and without reperfusion. Naunyn-Schmiedeberg's Arch Pharmacol 328:288–294

Böhm M, Gierschik P, Jakobs KH, Pieske B, Schnabel P, Ungerer M, Erdmann E (1990) Increase of $G_{i\alpha}$ in human hearts with dilated but not ischemic cardiomyopathy. Circulation 82:1249–1265

Böhm M, Eschenhagen T, Gierschik P, Larisch K, Lensche H, Mende U, Schmitz W, Schnabel P, Scholz H, Steinfath M, Erdmann H (1994) Radioimmunochemical quantification of $G_{i\alpha}$ in right and left ventricles from patients with ischemic and dilated cardiomyopathy and predominant left ventricular failure. J Mol Cell Cardiol 26:133–149

Chen L, Chen CX, Gan XT, Beier N, Scholz W, Karmazyn M (2004) Inhibition and reversal of myocardial infarction-induced hypertrophy and heart failure by NHE-1 inhibition. Am J Physiol 286:H381–H387

Chiariello M, Brevetti G, DeRosa G, Acunzo F, Petillo F, Rengo F, Condorelli M (1980) Protective effects of simultaneous alpha and beta adrenergic receptor blockade on myocardial cell necrosis after coronary arterial occlusion in rats. Am J Cardiol 46:249–254

Colatsky TH (1989) Models of myocardial ischemia and reperfusion injury: role of inflammatory mediators in determining infarct size. In: Pharmacological methods in the control of inflammation. Alan R. Liss, New York, pp 283–320

Fishbein MC, MacLean D, Maroko PR (1978) Experimental myocardial infarction in the rat. Qualitative and quantitative changes during pathologic evolution. Am J Pathol 90:57–70

Fishbein MC, Hare AC, Gissen SA, Spadaro J, MacLean D, Maroko PR (1980) Identification and quantification of histochemical border zones during the evolution of myocardial infarction in the rat. Cardiovasc Res 14:41–49

Flaim SF, Zelis R (1981) Diltiazem pretreatment reduces experimental myocardial infarct size in rat. Pharmacology 23:281–286

Gargiulo S, Greco A, Gramanzini M, Petretta MP, Ferro A, Larobina M, Panico M, Brunetti A, Cuocolo A (2012) PET/CT imaging in mouse models of myocardial ischemia. J Biomed Biotechnol 2012:541872

Gomoll AW, Lekich RF (1990) Use of the ferret for a myocardial ischemia/salvage model. J Pharmacol Methods 23:213–223

Gould KE, Taffet GE, Michael LH, Christie RM, Konkol DL, Pocius JS, Zachariah JP, Chaupin DF, Daniel SL, Sandusky GE Jr, Hartley CJ, Entman ML (2001) Heart failure and greater infarct expansion in middle-aged mice: a relevant model for postinfarction failure. Am J Physiol 282:H615–H621

Guo Y, Wu WJ, Qiu Y, Tang XL, Yang Z, Bolli R (1998) Demonstration of an early and a late phase of ischemic preconditioning in mice. Am J Physiol 275: H1375–H1387

Guo Y, Stein AB, Wu WJ, Zhu X, Tan W, Li Q, Bolli R (2005) Late preconditioning induced by NO donors, adenosine A_1 receptor agonists, and δ_1-opioid receptor agonists is mediated by iNOS. Am J Physiol 289: H2251–H2257

Innes IR, Weisman H (1981) Reduction in the severity of myocardial infarction by sulfinpyrazone. Am Heart J 102:153–157

Janssens S, Pokreisz P, Schoonjans L, Pellens M, Vermeersch P, Tjwa M, Jans P, Scherrer-Crosbie M, Picard MH, Szelid Z, Gillijns H, Van de Werf F, Collen D, Bloch KD (2004) Cardiomyocyte-specific overexpression of nitric oxide synthase 3 improves left ventricular performance and reduces compensatory hypertrophy after myocardial infarction. Circ Res 94:1256–1262

Johns TNP, Olson BJ (1954) Experimental myocardial infarction. I. A method of by coronary occlusion in small animals. Ann Surg 140:675–682

Jones SP, Lefer DJ (2001) Cardioprotective actions of acute HMG-CoA reductase inhibition in the setting of myocardial infarction. Acta Physiol Scand 173:139–143

Kajstura J, Zhang X, Reiss K, Szoke E, Li P, Lagastra C, Cheng W, Darzynkiewicz Z, Olivetti G, Anversa P (1994) Myocyte cellular hyperplasia and myocyte cellular hypertrophy contribute to chronic ventricular remodeling in coronary artery narrowing-induced cardiomyopathy in rats. Circ Res 74:382–400

Kanno S, Kovacs A, Yamada KA, Saffitz JE (2003) Connexin43 as a determinant of myocardial infarct size following coronary occlusion in mice. J Am Coll Cardiol 41:681–686

Kaufman N, Gavan TL, Hill RW (1959) Experimental myocardial infarction in the rat. Arch Pathol 57:482–488

Kim DY, Kim HJ, Yu KH, Min JJ (2012) Synthesis of 18F-labeled (2-(2-fluoroethoxy)ethyl) triphenylphosphonium cation as a potential agent for myocardial imaging using positron emission tomography. Bioorg Med Chem Lett 22:319–322

Kim DY, Kim HS, Jang HY, Kim JH, Bom HS, Min JJ (2014) Comparison of the cardiac microPET images obtained using [(18)F]FPTP and [(13)N]NH3 in rat myocardial infarction models. ACS Med Chem Lett 5(10):1124–1128

Kouchi I, Zolk O, Jockenhövel F, Itter G, Linz W, Cremers B, Böhm M (2000) Increase in $G_{i\alpha}$ protein accompanies progression of post-infarction remodelling in hypertensive cardiomyopathy. Hypertension 36:42–47

Kuhlmann MT, Kirchhof P, Klocke R, Hasib L, Stypmann J, Fabritz L, Stelljes M, Tian W, Zwiener M, Mueller M, Kienast J, Breithardt G, Nikol S (2006) G-CSF/SCF reduces inducible arrhythmias in the infracted heart potentially via increased connexin43 expression and arteriogenesis. J Exp Med 203:87–97

LaPointe MC, Mendez M, Leung A, Tao Z, Yang XP (2004) Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse. Am J Physiol 286:H1416–H1424

Le Meur Y, Aldigier JC, Praloran V (1998) Is plasma Ac-SDKP level is a reliable marker of chronic angiotensin-converting enzyme inhibition in hypertensive patients? Hypertension 31:1201–1202

Leprán I, Koltai M, Szekeres L (1981) Effect of non-steroid antiinflammatory drugs in experimental myocardial infarction in rats. Eur J Pharmacol 69:235–238

Linz W, Wiemer G, Schmidts HL, Ulmer W, Ruppert D, Schölkens BA (1996) ACE inhibition decreases post-operative mortality in rats with left ventricular hypertrophy and myocardial infarction. Clin Exp Hypertens 18:691–712

Liu YH, Yang XP, Nass O, Sabbah HN, Peterson E, Carretero OA (1997) Chronic heart failure induced by coronary artery ligation in Lewis inbred rats. Am J Physiol 272(2 Pt2):H722–H727

Lutgens E, Daemen MJ, de Muinck ED, Debets J, Leenders P, Smits JF (1999) Chronic myocardial infarction in the mouse: cardiac structural and functional changes. Cardiovasc Res 41:586–593

MacLean D, Fishbein MC, Braunwald E, Maroko PR (1978) Long-term preservation of ischemic myocardium after experimental coronary artery occlusion. J Clin Invest 61:541–551

Michael LH, Entman ML, Hartley CJ, Youker KA, Zhu J, Hall SR, Hawkins HK, Berens K, Balantyne CM (1995) Myocardial ischemia and reperfusion: a murine model. Am J Physiol 269(Heart Circ Physiol 38): H2147–H2154

Michael LH, Ballantyne CM, Zachariah JP, Gould KE, Pocius JS, Taffet GE, Hartley CJ, Pham TT, Daniel SL, Funk E, Entman ML (1999) Myocardial infarction and remodeling in mice: effect of reperfusion. Am J Physiol 277:H660–H668

Nguyen T, Salibi EE, Rouleau JL (1998) Postinfarction survival and inducibility of ventricular arrhythmias in the spontaneously hypertensive rat. Effects of ramipril and hydralazine. Circulation 98:2074–2080

Pfeffer JM, Pfeffer MA, Braunwald E (1985) Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ Res 57:84–95

Porzio S, Masseroli M, Messori A, Forloni G, Olivetti G, Jeremic G, Riva E, Luvara G, Latini R (1995) A simple, automatic method for morphometric analysis of the left ventricle in rats with myocardial infarction. J Pharmacol Toxicol Methods 33:221–229

Rasoul S, Carretero OA, Peng H, Cavasin MA, Zhuo J, Sanchez-Mendoza A, Brigstock DR, Rhaleb NE (2004) Antifibrotic effect of Ac-SDKP and angiotensin converting enzyme inhibition in hypertension. J Hypertens 22:593–603

Roberts CS, MacLean D, Braunwald E, Maroko PR, Kloner RA (1983) Topographic changes in the left ventricle after experimentally induced myocardial infarction in the rat. Am J Cardiol 51:873–876

Sakai K, Akima M, Aono J (1981) Evaluation of drug effects in a new experimental model of angina pectoris in the intact anesthetized rat. J Pharmacol Methods 5:325–336

Scherrer-Crosbie M, Steudel W, Ullrich R, Hunziker PR, Liel-Cohen N, Newell J, Zaroff J, Zapol WM, Picard MH (1999) Echocardiographic determination of risk area in a murine model of myocardial ischemia. Am J Physiol 277:H986–H992

Scholz W, Albus U, Counillon L, Gögelein H, Lang HJ, Linz W, Weichert A, Schölkens BA (1995) Protective effects of HOE 642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischemia and reperfusion. Cardiovasc Res 29:260–268

Selye H, Bajusz E, Grasso S, Mendell P (1960) Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 11:398–407

Shibuya K, Kanasaki K, Isono M, Sato H, Omata M, Sugimoto T, Araki SI, Isshiki K, Kashiwagi A, Haneda M, Koya D (2005) *N*-Acetyl-seryl-aspartyl-lysine-proline prevents renal insufficiency and matrix expansion in diabetic d*b*/d*b* mice. Diabetes 54:838–845

Sia YT, Lapointe N, Parker TG, Tsoporis JN, Deschepper CP, Calderose A, Pourdjabbar A, Jasmin JF, Sarrazin JF, Liu P, Asam A, Butany J, Rouleau JL (2002) Beneficial effects of long-term use of the antioxidant probucol in heart failure in the rat. Circulation 105:2549–2555

Teunissen BEJ, Jongsma HJ, Bierhuizen MFA (2004) Regulation of myocardial connexins during hypertrophic remodelling. Eur Heart J 25:1979–1989

Walker MJA, MacLeod BA, Curtis MJ (1991) Myocardial ischemia and infarction. Comp Pathol Bull 23:3–4

Weinberg EO, Scherrer-Crosbie M, Picard MH, Nasseri BA, MacGillivray C, Gannon J, Lian Q, Bloch KD, Lee RT (2005) Rosuvastatin reduces experimental left ventricular infarct size after ischemia-reperfusion injury but not total coronary occlusion. Am J Physiol 288:H1802–H1809

Yang XP, Sabbah HN, Liu YH, Sharov VG, Mascha EJ, Alwan I, Carretero OA (1992) Ventriculographic evaluation in three rat models of cardiac dysfunction. Am J Physiol 265(6 Pt 2):H1946–H1952

Yang F, Yang XP, Liu YH, Xu J, Cingolani O, Rhaleb NE, Carretero OA (2004) Ac-SDKP reverses inflammation and fibrosis in rats with heart failure after myocardial infarction. Hypertension 43:229–236

Yang Z, Day YJ, Toufektsian MC, Ramos SI, Marshall M, Wang XQ, French BA, Linden J (2005) Infarct-sparing effect of A_{2A}-adenosine receptor activation is due primarily to its action on lymphocytes. Circulation 111:2190–2197

Ytrehus K, Liu Y, Tsuchida A, Miura T, Liu GS, Yang XM, Herbert D, Cohen MV, Downey JM (1994) Rat and rabbit heart infarction: effects of anesthesia, perfusate, risk zone, and method of infarct sizing. Am J Physiol 267(Heart Circ Physiol 36):H2383–H2390

Occlusion of Coronary Artery in Anesthetized Dogs and Pigs

Abendroth RR, Meesmann W, Stephan K, Schley G, Hübner H (1977) Effects of the β-blocking agent Atenolol on arrhythmias especially ventricular fibrillation and fibrillation threshold after acute experimental coronary artery occlusion. Z Kardiol 66:341–350

Black SC, Gralinski MR, Friedrichs GS, Kilgore KS, Drsicoll EM, Lucchesi BR (1995) Cardioprotective effects of heparin or *N*-acetylheparin in an *in vivo* model of myocardial and ischemic reperfusion injury. Cardiovasc Res 29:629–636

Chiariello M, Gold HL, Leinbach RC, Davis MA, Maroko PR (1976) Comparison between the effects of nitroprusside and nitroglycerin on ischemic injury during acute myocardial infarction. Circulation 54:766–773

Etoh T, Joffs C, Deschamps AM, Davis J, Dowdy K, Hendrick J, Baicu S, Mukherjee R, Manhaini M, Spinale FG (2001) Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs. Am J Physiol 281:H987–H994

Garcia-Dorado D, González MA, Barrabés JA, Ruiz-Meana M, Solares J, Lidon RM, Blanco J, Puigfel Y, Piper HM, Soler-Soler J (1997) Prevention of ischemic rigor contracture during coronary occlusion by inhibition of Na^+-H^+ exchange. Cardiovasc Res 35:80–89

Hartman JC, Warltier DC (1990) A model of multivessel coronary artery disease using conscious, chronically instrumented dogs. J Pharmacol Methods 24:297–310

Holmborn B, Näslund U, Eriksson A, Virtanen I, Thornell LE (1993) Comparison of triphenyltetrazolium chloride (TTC) staining versus detection of fibronectin in experimental myocardial infarction. Histochemistry 99:265–275

Klein HH, Pich S, Bohle RM, Wollenweber J, Nebendahl K (1995) Myocardial protection by Na^+/H^+ exchange inhibition in ischemic, reperfused porcine hearts. Circulation 92:912–917

Klein HH, Bohle RM, Pich S, Lindert-Heimberg S, Wollenweber J, Nebendahl K (1997) Time delay of cell death by Na^+/H^+ exchange inhibition in regionally ischemic, reperfused porcine hearts. J Cardiovasc Pharmacol 30:235–240

Li T, Wei X, Watkins AC, Sanchez PG, Wu ZJ, Griffith BP (2013) Prophylactic amiodarone and lidocaine improve survival in an ovine model of large size myocardial infarction. J Surg Res 185(1):152–158

Lukács E, Magyari B, Tóth L, Petrási Z, Repa I, Koller A, Horváth I (2012) Overview of large animal myocardial infarction models. Acta Physiol Hung 99(4):365–381

Lukács E, Magyari B, Tóth L, Petneházy Ö, Petrási Z, Simor T, Gyöngyösi M, Repa I, Koller Á, Rőth E, Horváth IG (2013) Evaluation of experimental myocardial infarction models via electromechanical mapping and magnetic resonance imaging. Can J Physiol Pharmacol 91(8):617–624

Martorana PA, Göbel H, Kettenbach B, Nitz RE (1982) Comparison of various methods for assessing infarct-size in the dog. Basic Res Cardiol 77:301–308

Martorana PA, Kettenbach B, Breipohl G, Linz W (1990) Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a bradykinin antagonist. Eur J Pharmacol 182:395–396

McCall FC, Telukuntla KS, Karantalis V, Suncion VY, Heldman AW, Mushtaq M, Williams AR, Hare JM (2012) Myocardial infarction and intramyocardial injection models in swine. Nat Protoc 7(8):1479–1496

Nachlas MN, Shnitka TK (1963) Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. Am J Pathol 42:379–396

Raberger G, Krumpl G, Mayer N (1986) A model of transient myocardial dysfunction in conscious dogs. J Pharmacol Methods 16:23–37

Reimer KA, Jennings RB, Cobb FR, Murdock RH, Greenfield JC, Becker LC, Bulkley BH, Hutchins GM, Schwartz RP, Bailey KR, Passamani ER (1985) Animal models for protecting ischemic myocardium: results of the NHLBI cooperative study. Comparison of unconscious and conscious dog models. Circ Res 56:651–665

Scherlag BJ, El-Sherif N, Hope R, Lazzara R (1974) Characterization and localization of ventricular arrhythmias resulting from myocardial ischemia and infarction. Circ Res 35:372–383

Schaper W, Frenzel H, Hort W (1979) Experimental coronary artery occlusion. I. Measurement of infarct size. Basic Res Cardiol 74:46–53

Spata T, Bobek D, Whitson BA, Parthasarathy S, Mohler PJ, Higgins RS, Kilic A (2013) A nonthoracotomy myocardial infarction model in an ovine using autologous platelets. Biomed Res Int 2013:938047

Symons JD, Correa SD, Schaefer S (1998) Na-H exchange inhibition with cariporide limits functional impairment due to repetitive ischemia. J Cardiovasc Pharmacol 32(6):853–862

Acute Ischemia by Injection of Microspheres in Dogs

Gorodetskaya EA, Dugin SF, Medvedev OS, Allabergenova AE (1990) A simple method to produce acute heart failure by coronary vessel embolization in closed chest rats with microspheres. J Pharmacol Methods 24:43–51

Rooke GA, Feigl EO (1982) Work as a correlate of canine left ventricular oxygen consumption, and the problem of catecholamine oxygen wasting. Circ Res 50:273–286

Schölkens BA, Martorana PA, Göbel H, Gehring D (1986) Cardiovascular effects of the converting enzyme inhibitor ramipril (Hoe 498) in anesthetized dogs with acute ischemic left ventricular failure. Clin Exp Theory Pract A8(6):1033–1048

Smiseth OA (1983) Effects of the β-adrenergic receptor agonist pirbuterol on cardiac performance during acute ischaemic left ventricular failure in dogs. Eur J Pharmacol 87:379–386

Smiseth OA, Mjøs OD (1982) A reproducible and stable model of acute ischemic left ventricular failure in dogs. Clin Physiol 2:225–239

Influence on Myocardial Preconditioning

Baxter GF, Marber MS, Patel VC, Yellon DM (1994) Adenosine receptor involvement in a delayed phase of myocardial protection 24 h after ischemic preconditioning. Circulation 90:2993–3000

Garcia-Dorado D, Théroux P, Elizaga J, Galiñanes M, Solares J, Riesgo M, Gomez MJ, Garcia-Dorado A, Aviles FF (1987) Myocardial reperfusion in the pig heart model: infarct size and duration of coronary occlusion. Cardiovasc Res 21:537–544

Gho BC, Schoemaker RG, van der Lee C, Sharma HS, Verdouw PC (1994) Myocardial infarct size limitation in rat by transient renal ischemia. Circulation 90:I-476/2557

Gross JG, Auchampach JA (1992) Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ Res 70:223–233

Hoff PT, Tamura Y, Lucchesi BR (1990) Cardioprotective effects of amlodipine on ischemia and reperfusion in two experimental models. Am J Cardiol 66:10H–16H

Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, Vogel M, Sorensen K, Redington AN, MacAllister R (2002) Transient limb ischemia induces remote ischemic preconditioning in vivo. Circulation 106:2881–2883

Li GC, Vasquez JA, Gallagher KP, Lucchesi BR (1990) Myocardial protection with preconditioning. Circulation 82:609–619

Linz W, Wiemer G, Schölkens BA (1992) ACE-inhibition induces NO-formation in cultured bovine endothelial cells and protects isolated ischemic rat hearts. J Mol Cell Cardiol 24:909–919

Linz W, Wiemer G, Gohlke P, Unger T, Schölkens BA (1994) Kardioprotektive Effekte durch Ramipril nach Ischämie und Reperfusion in tierexperimentellen Studien. Z Kardiol 83(Suppl 4):53–56

Liu GS, Thornton J, Van Winkle DM, Stanley WH, Olsson RA, Downey JM (1991) Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 84:350–356

Merlocco AC, Redington KL, Disenhouse T, Strantzas SC, Gladstone R, Wei C, Tropak MB, Manlhiot C, Li J, Redington AN (2014) Transcutaneous electrical nerve stimulation as a novel method of remote preconditioning: in vitro validation in an animal model and first human observations. Basic Res Cardiol 109(3):406

Mickelson JK, Simpson PJ, Lucchesi BR (1989) Streptokinase improves reperfusion blood flow after coronary artery occlusion. Int J Cardiol 23:373–384

Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischaemia: a delay of lethal cell injury in ischaemic myocardium. Circulation 74:1124–1136

Parratt JR (1994) Protection of the heart by ischemic preconditioning: mechanisms and possibilities for pharmacological exploitation. TIPS 15:19–25

Parratt J, Vegh A (1994) Pronounced antiarrhythmic effects of ischemic preconditioning. Cardioscience 5:9–18

Shimizu M, Tropak M, Diaz RJ, Suto F, Surendra H, Kuzmin E, Li J, Gross G, Wilson GJ, Callahan J, Redington AN (2009) Transient limb ischaemia remotely preconditions through a humoral mechanism acting directly on the myocardium: evidence suggesting cross-species protection. Clin Sci (Lond) 117:191–200

Simpson PJ, Fantone JC, Mickelson JK, Gallagher KP, Lucchesi BR (1988) Identification of a time window for therapy to reduce experimental canine myocardial injury: suppression of neutrophil activation during 72 h of reperfusion. Circ Res 63:1070–1079

Sun JZ, Tang XL, Knowton AA, Park SW, Qiu Y, Bolli R (1995) Late preconditioning against myocardial stunning. An endogenous protective mechanism that confers resistance to post-ischemic dysfunction 24 h after brief ischemia in conscious pigs. J Clin Invest 95:388–403

Szekeres L, Szilvássy Z, Fernandy P, Nagy I, Karcsu S, Scáti S (1997) Delayed cardiac protection against harmful consequences of stress can be induced in experimental atherosclerosis in rabbits. J Mol Cell Cardiol 29:1977–1983

Szilvássy Z, Fernandy P, Bor P, Jakab I, Lonovics J, Koltai M (1994) Ventricular overdrive pacing-induced antiischemic effect: a conscious rabbit model of preconditioning. Am J Physiol 266(Heart Circ Physiol 35):H2033–H2041

Szilvássy Z, Fernandy P, Szilvássy J, Nagy I, Karcsu S, Lonovics J, Dux L, Koltai M (1995) The loss of pacing-induced preconditioning in atherosclerotic rabbits: role of hypercholesterolaemia. J Mol Cell Cardiol 27:2559–2569

Tamura Y, Chi L, Driscoll EM, Hoff PT, Freeman BA, Gallagher KP, Lucchesi BR (1988) Superoxide dismutase conjugated to polyethylene glycol provides sustained protection against myocardial ischemia/reperfusion injury in canine heart. Circ Res 63:944–959

Toombs CF, McGee DS, Johnston WE, Vinten-Johansen J (1993) Protection from ischaemic-reperfusion injury with adenosine pretreatment is reversed by inhibition of ATP sensitive potassium channels. Cardiovasc Res 27:623–629

van Gilst WH, de Graeff PA, Wesseling H, de Langen CDJ (1986) Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors: a comparison of captopril, enalapril, and HOE 498. J Cardiovasc Pharmacol 8:722–728

Vegh A, Szekeres L, Parrat JR (1990) Protective effects of preconditioning of the ischaemic myocardium involve cyclooxygenase products. Cardiovasc Res 24:1020–1023

Volovsek A, Subramanian R, Reboussin D (1992) Effects of duration of ischaemia during preconditioning on mechanical function, enzyme release and energy production in the isolated working rat heart. J Mol Cell Cardiol 24:1011–1019

Wiemer G, Schölkens BA, Becker RHA, Busse R (1991) Ramiprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium-derived bradykinin. Hypertension 18:558–563

Yang XM, Baxter GF, Heads RJ, Yellon DM, Downey JM, Cohen MV (1996) Infarct limitation of the second window of protection in a conscious rabbit model. Cardiovasc Res 31:777–783

Yao Z, Gross GJ (1994) A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course, and role of K_{ATP} channels. Circulation 89:1229–1236

MRI Studies of Cardiac Function

Al-Shafei AIM, Wise RG, Gresham GA, Bronns G, Carpenter TA, Hall LD, Huang CHL (2002a) Non-invasive magnetic resonance assessment of myocardial changes and the effects of angiotensin-converting enzyme inhibition in diabetic rats. J Physiol (Lond) 538:541–553

Al-Shafei AIM, Wise RG, Gresham GA, Carpenter TA, Hall LD, Huang CHL (2002b) Magnetic resonance imaging analysis of cardiac cycle events in diabetic rats: the effect of angiotensin-converting enzyme inhibition. J Physiol (Lond) 538:555–572

Ballon D, Graham MC, Midownik S, Koutcher JA (1990) A 64 MHz half-birdcage resonator for clinical imaging. J Magn Reson 90:131–140

Bryant D, Becker L, Richardson J, Shelton J, Franco F, Peshock R, Thompson M, Giroir B (1998) Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor-α. Circulation 97:1375–1381

Franco F, Thomas GD, Giroir B, Bryant D, Bullock MC, Chwialkowski MC, Victor RG, Peshock RM (1999) Magnetic resonance imaging and invasive evaluation of development of heart failure in transgenic mice with myocardial expression of tumor necrosis factor-α. Circulation 99:448–454

Itter G, Jung W, Juretschke P, Schölkens BA, Linz W (2004c) A model of chronic heart failure in spontaneous hypertensive rats (SHR). Lab Anim 38:138–146

Kraitchman DL, Sampath S, Castillo E, Derbyshire JA, Boston RC, Bluemke DA, Gerber BL, Pronce JL, Osman NF (2003) Quantitative ischemia detection during cardiac magnetic resonance stress testing by use of FastHARP. Circulation 107:2025–2030

Pelzer T, Jazbutyte V, Hu K, Segerer S, Nahrendorf M, Nordbeck P, Bonz AW, Muck J, Fritzemeier KH, Hegele-Hartung C, Ertl G, Neyses L (2005) The estrogen receptor-α agonist 16α-LE2 inhibits cardiac hypertrophy and improves hemodynamic function in estrogen-deficient spontaneously hypertensive rats. Cardiovasc Res 67:604–612

Peshock RM, Willet DL, Sayad DE, Hundley WG, Chwialkowski MC, Clarke GD, Parkey RW (1996) Quantitative MR imaging of the heart. Magn Reson Imaging Clin N Am 4:267–305

Reddy VY, Malchano ZJ, Holmvang G, Schmidt EJ, d'Avila A, Houghtaling C, Chan RC (2004) Integration of cardiac magnetic resonance imaging with three-dimensional electroanatomic mapping to guide left ventricular catheter manipulation. Feasibility of a porcine model of healed myocardial infarction. J Am Coll Cardiol 44:2202–2213

Wiesmann F, Frydrychowicz A, Rautenberg J, Illinger R, Rommel E, Haase A, Neubauer S (2002) Analysis of right ventricular function in healthy mice and a murine model of heart failure by in vivo MRI. Am J Physiol 283:H1065–H1071

MRI Studies After Heart and Lung Transplantation

Dodd SJ, Williams M, Suhan JP et al (1999) Detection of single mammalian cells by high-resolution magnetic resonance imaging. Biophys J 76:103–109

Gellissen J, Axmann C, Prescher A et al (1999) Extra- and intracellular accumulation of ultrasmall superparamagnetic iron oxides (USPIO) in experimentally induced abscesses of the peripheral soft tissues and their effects on magnetic resonance imaging. Magn Reson Imaging 17:557–567

Kanno S, Lee PC, Dodd SJ et al (2000) A novel approach using magnetic resonance imaging for the detection of lung allograft rejection. J Thorac Cardiovasc Surg 120:923–934

Kanno S, Wu YJ, Lee PC, Dodd SJ, Williams M, Griffith HP, Ho C (2001) Macrophage accumulation associated with rat cardiac allograft rejection detected by magnetic resonance imaging with ultrasmall superparamagnetic iron oxide particles. Circulation 104:934–938

Palmacci S, Josephson L (1993) Synthesis of polysaccharide covered superparamagnetic oxide colloids. US Patent 5,262,176 Example 1, 16 Nov 1993

Plastic Casts from Coronary Vasculature Bed

Boor PJ, Reynolds ES (1977) A simple planimetric method for determination of left ventricular mass and necrotic myocardial mass in postmortem hearts. Am J Clin Pathol 68:387–392

Kadatz R (1969) Sauerstoffdruck und Durchblutung im gesunden und koronarinsuffizienten Myocard des Hundes und ihre Beeinflussung durch koronarerweiternde Pharmaka. Arch Kreislaufforsch 58:263–293

Kadatz R (1971) Agents acting on coronary blood vessels. In: Turner RA, Hebborn P (eds) Screening methods in pharmacology, vol II. Academic Press, New York/London, pp 41–60

Lumb G, Hardy LB. (1963) Collateral circulation in the heart. N C Med J. 24:456–60.

Meesman W (1982) Early arrhythmias and primary ventricular fibrillation after acute myocardial ischemia in relation to pre-existing collaterals. In: Parratt JR (ed) Early arrhythmias resulting from myocardial ischemia. Mechanisms and prevention by drugs. McMillan, London, pp 93–112

Meesmann W, Bachmann GW (1966) Pharmakodynamisch induzierte Entwicklung von Koronar-Kollateralen in Abhängigkeit von der Dosis. Arzneim Forsch 16:501–509

Meesmann W, Schulz FW, Schley G, Adolphsen P (1970) Überlebensquote nach akutem experimentellem Coronarverschluß in Abhängigkeit von Spontankollateralen des Herzens. Z Ges Exp Med 153:246–264

Schaper W, Xhonneux R, Jageneau AHM (1965) Stimulation of the coronary collateral circulation by Lidoflazine (R 7904). Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 252:1–8

Schmidt HD, Schmier J (1966) Eine Methode zur Herstellung anatomischer Korrosionspräparate – dargestellt am Koronargefäßsystem des Hundes. Zschr Kreislaufforsch 55:297–305

Vineberg AM, Chari RS, Pifarré R, Mercier C (1962) The effect of Persantin on intercoronary collateral circulation and survival during gradual experimental coronary occlusion. Can Med Assoc J 87:336–345