Cardiovascular Analysis In Vivo

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Hemodynamic Screening in Anesthetized Rats

Purpose and Rationale

The test is used to detect the effect of compounds on blood pressure and heart rate of anesthetized rats and to check for possible interference with adrenergic receptors. Antihypertensive agents with different mechanisms of action can be detected with this test.

Procedure

Male Sprague–Dawley rats weighing 250–400 g are used. At least two animals are necessary for screening of one compound. The rats are anesthetized by intraperitoneal injection of 8 ml/kg of a solution of 8 % urethane and 0.6 % chloralose. The trachea is cannulated to facilitate spontaneous respiration. Body temperature is maintained at 38 °C by placing the animal on a heating pad.

The left femoral vein is cannulated for drug administration, which is standardized to injections of 0.2 ml/100-g body weight over a period of 1 min. For measurement of hemodynamic parameters and for intra-arterial administration of test compound, a cannula is inserted retrogradely into the right carotid artery. The tip of the catheter is positioned close to the origin of the subclavian artery. This allows most of the injected substances to reach the CNS via the vertebral artery before going into the general circulation.

For continuous monitoring of blood pressure (systolic and diastolic pressure) and heart rate, the catheter is connected to a pressure transducer (Statham DB 23).

When stable hemodynamic conditions are achieved for at least 20 min (control values), test boli of adrenaline (1 mg/kg) and isoprenaline (0.25 mg/kg) are administered. When baseline values are again established, increasing doses of the test substance (0.01, 0.1, 3.0 mg/kg) are given intra-arterially. In case of no effect, the interval between successive doses is 15 min, otherwise 60 min. To check for α- or β-blocking activity,

adrenaline and isoprenaline administration is repeated after injection of the highest dose of test compound. If the test compound shows no effect, a standard antihypertensive compound is administered for control purpose.

Hemodynamic parameters are recorded continuously during the whole experiment.

Evaluation

Changes in blood pressure and heart rate after drug administration are compared to control values obtained during the 20-min predrug period.

Maximal changes in BP and HR and duration of the effect are reported.

The results are scored relative to the efficacy of standard compounds for the degree of the effect and the duration of the effect.

Statistical significance is not tested because of the small number of animals used ($n = 2$, sometimes 3 or 4), but larger numbers of animals have to be used for quantitative evaluation.

Critical Assessment of the Method

Due to the administration of the test compounds via the right common carotid artery, not only peripherally acting vasodilators and neuron blockers but also compounds affecting the blood pressure regulating mechanisms in the CNS are detected. Bolus injections of adrenaline and isoprenaline reveal possible α- or β-antagonistic effects.

Standard data:

The following compounds at the doses indicated lead to a strong decrease in blood pressure:

- Clonidine 0.008 mg/kg
- Dihydralazine 1.0 mg/kg
- Phentolamine 3.0 mg/kg
- Prazosin 0.1 mg/kg
- Propranolol 1.0 mg/kg
- Urapidil 1.0 mg/kg
- Verapamil 0.1 mg/kg

Modifications of the Method

Several authors (Mervaala et al. 1999; Wallerath et al. 1999; Rothermund et al. 2000; Baltatu et al. 2001) monitored arterial pressure and heart rate using a pressure transducer system and continuously recorded on a computer-based registration system (TSE, Bad Homburg, Germany).

A procedure for differential intra-arterial pressure recordings from different arteries in the rat was described by Pang and Chan (1985).

DeWildt and Sangster (1983) described the evaluation of derived aortic flow parameters measured by means of electromagnetic flowmetry as indices of myocardial contractility in anesthetized rats.

Using a special Millar ultraminiature catheter pressure transducer and a thermodilution microprobe, Zimmer et al. (1987, 1988) measured right ventricular functional parameters in anesthetized, closed-chest rats.

Veelken et al. (1990) published improved methods for baroreceptor investigations in chronically instrumented rats.

Salgado and Krieger (1988), de Abreu and Salgado (1990), and Da Silva et al. (1994) studied the function of the **baroreceptor reflex** in thiopental anesthetized rats. The left aortic nerve was isolated and supported by a bipolar stainless steel electrode and carefully insulated with silicone rubber. Carotid pressure was recorded simultaneously with aortic nerve discharges on an oscilloscope and monitored with a loudspeaker.

King et al. (1987) developed a cross circulation technique in rats to distinguish central from peripheral cardiovascular actions of drugs. The right common carotid arteries were ligated, and the left common carotid arteries and left and right external jugular veins of two phenobarbital-anesthetized rats were connected with polyethylene tubing so that peripheral blood from one rat, A, supplied the head of another rat, B, and then returned to the body of A, and vice versa, for peripheral blood from rat B. Each rat was artificially ventilated with O_2, the chest was opened, and both subclavian arteries were ligated. Prior to the ligation of the subclavian arteries, blood flow from rat A supplied its own brain and both brain

hemispheres but not the brain stem of rat B. Following subclavian artery ligation, blood flow from rat A did not supply A's brain, but supplied both hemispheres and brain stem of rat B. The head of each rat was, therefore, rendered dependent on the carotid arterial blood supply from another rat. This rat cross circulation preparation can be used to separate the central and peripheral cardiovascular actions of drugs.

Zavisca et al. (1994) studied the hypertensive responses to defined electrical and mechanical stimuli in anesthetized rats. Rats were given etomidate, 3.8 mg/kg/h intravenously following carotid artery and jugular vein cannulation. At 15 min after beginning the infusion, four types of noxious stimuli were administered sequentially at 1-min intervals: type 1 (square electrical waves 125 cps, 1.6 ms, 2-s duration, varying current from 0.4 to 12 mA), type 2 (a single 10-mA electrical stimulus, 5-s train duration), type 3 (tail clamping), and type 4 (skin incision). After each stimulus, maximum change in systolic blood pressure was measured. Graded electrical stimulation allowed the best quantitative evaluation of the hypertensive response to noxious stimuli.

Hyman et al. (1998) described a novel catheterization technique for the in vivo measurements of **pulmonary vascular responses** in rats. Male Charles River rats weighing 26–340 g were anesthetized and strapped in supine position to a fluoroscopic table. They breathed air enriched with oxygen through an endotracheal tube inserted by tracheostomy. Catheters were inserted into the femoral blood vessels. The venous catheters were passed to the right atrium under fluoroscopy. A F-1 thermistor–catheter was passed from the left carotid artery into the ascending aorta under fluoroscopy, and a PE-50, 150-mm plastic catheter with a specially constructed curved tip was passed fluoroscopically from the left jugular vein into the main pulmonary artery. A plastic radiopaque 22-gauge catheter 100 mm in length with a curved tip was passed with a 0.025-mm soft-tip coronary guiding catheter from the right jugular vein through the right atrium to the inferior vena cava. The coronary soft-tip guide was then withdrawn. A specially curved 102.5-mm transseptal needle, 0.4 mm in diameter, was then passed though the catheter. Both the needle and catheter were withdrawn into the superior portion of the right atrium under fluoroscopic guidance so that the needle and catheter both rotated freely. With the rat in a slight left anterior oblique position, the catheter and needle were carefully rotated anteriorly to the intra-atrial septum. With gentle pressure, the catheter and needle can be felt and seen fluoroscopically to pass through the atrial septum. As the needle was withdrawn, the curve of the catheter permitted passage of the tip into the vein draining either the left or right lower lobe. The catheter was carefully positioned near the pulmonary venoatrial junction and fixed in place. Mean pressures in the femoral artery, pulmonary artery, and pulmonary vein at the venoatrial junction were measured with pressure transducers and recorded on a polygraph. Cardiac output was obtained in triplicate by delivering 0.1 ml normal saline at room temperature into the femoral venous catheter at the right venoatrial junction and determining thermodilution cardiac output with the thermistor–catheter in the ascending aorta.

Hayes (1982) described a technique for determining contractility, intraventricular pressure, and heart rate in the **anesthetized guinea pig** by inserting a needle, attached to a pressure transducer, through the chest wall into the left ventricle.

Williams et al. (1965) used **castrated male ferrets** anesthetized by intramuscular injection of a mixture of 55 mg/kg ketamine and 4 mg/kg xylazine to measure the effects of a nonpeptidyl endothelin antagonist on endothelin-induced pressor responses.

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Blood Pressure in Pithed Rats

Purpose and Rationale

The pithed rat has been proposed for assessing pressor substances by Shipley and Tilden (1947). The preparation is frequently used to evaluate drug action on the cardiovascular system since this preparation is devoid of neurogenic reflex control that may otherwise modulate the primary drug effect.

Procedure

Male rats weighing 250–350 g are prepared for pithing under halothane anesthesia. The left carotid artery is cannulated for blood pressure monitoring and blood sampling. Furthermore, the trachea and the right jugular vein are cannulated. The rats are pithed inserting a steel rod, 2.2 mm in diameter and about 11 cm in length, through the orbit and foramen magnum down the whole length of the spinal canal. Via the tracheotomy tube, the animals are ventilated with a small animal ventilation pump. Inspirated air is oxygen-enriched by providing a flow of oxygen across a T-piece attached to the air intake of the ventilation pump (Harvard Apparatus model 680). The rats are ventilated at a frequency of 60 cycles/min with a tidal volume of 2 ml/100 g body weight. Thirty minutes after pithing, a 0.3-ml blood sample is withdrawn from the carotid cannula and immediately analyzed for pO_2, pCO_2, pH, and derived bicarbonate concentration using an automatic blood gas analyzer. By alterations of the respiratory stroke volume of the pump, the values are adjusted to: pCO_2 30–43 mmHg, pH 7.36–7.50, and pO_2 87–105 mmHg.

Continuous registration of blood pressure and cardiac frequency (Hellige He 19 device and Statham P23 DB transducer) is performed via the left carotid artery.

In order to measure α_1- and α_2-antagonism, first dose–response curves are registered using doses of 0.1–30 μg/kg i.v. phenylephrine (a selective α_1-agonist) and 1–1,000 μg/kg i.v. BHT 920 (a selective α_2-agonist). The test drug is administered intravenously and the agonist dose–response curves are repeated again 15 min later.

Evaluation

If the curve of blood pressure response to the agonists is shifted, dose–response curves are plotted on a logarithmic probit scale and potency ratios are calculated.

Modifications of the Method

Gillespie and Muir (1967) described a method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat by coating those parts of the pithing rod which lay in the sacral and cervical region of the spinal cord with high resistance varnish to restrict stimulation to the thoracolumbar region. The steel rod is insulated with an adhesive throughout its length except for a 5-cm section which provides a sufficient stimulation area of the lower thoracolumbar nerves. For stimulating nerve fibers supplying exclusively the heart, a pithing rod is used which is insulated throughout its length except for a 0.5-cm section 7 cm proximal to the tip. The spinal cord is stimulated electrically using the pithing rod as the cathode and a hypodermic needle which is inserted under the skin near the right hind limb, as the anode. Varying the intensity and/or the duration of the stimulation, dose–response curves can be registered which are altered after treatment with drugs.

Curtis et al. (1986) described an improved pithed rat method by mounting the preparation vertically with the head pointing downwards resulting in considerably higher blood pressure and heart rate.

MacLean and Hiley (1988) studied the effect of artificial respiratory volume on the cardiovascular responses to an α_1- and α_2-adrenoceptor agonist in the air-ventilated pithed rat using microsphere technique and analysis of arterial blood gases and pH.

Trolin (1975) used decerebrated rats to study the clonidine-induced circulatory changes.

Balt et al. (2001) compared the angiotensin II type 1 (AT_1) receptor blockers losartan, irbesartan, telmisartan, and the ACE inhibitor captopril on inhibition of angiotensin-II-induced facilitation of sympathetic neurotransmission in the pithed rat.

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Antihypertensive Vasodilator Activity in Ganglion-Blocked, Angiotensin II-Supported Rats

Purpose and Rationale

The method is used to demonstrate direct vasodilator activity of potential antihypertensive agents. The experimental model is an anesthetized, ganglion-blocked rat whose blood pressure is maintained by an intravenous infusion of angiotensin II. The test allows to differentiate between centrally acting antihypertensives and peripheral vasodilators.

Procedure

Male Wistar rats weighing 275–450 g are anesthetized with a combination of urethane (800 mg/kg) and chloralose (60 mg/kg) administered intraperitoneally in a volume of 10 ml/kg. Following induction of anesthesia, chlorisondamine (2.5 mg/kg) is injected into the peritoneal cavity to abolish sympathetic and parasympathetic nerve activity. The right femoral artery is cannulated to monitor blood pressure (Statham pressure transducer P23 DB) and heart

rate. Both femoral veins are cannulated to administer drugs or infuse angiotensin II. The trachea is intubated and animals are allowed to breathe spontaneously. Following a stabilization interval of 10–15 min, angiotensin II is infused at a rate of 0.25 or 3.5 μg/min in a volume equivalent to 0.05 ml/min (Harvard infusion pump).

After an increase of blood pressure, a new elevated steady-state pressure is established within 15–20 min. Drugs are subsequently injected intravenously over an interval of 3 min in a volume of 2 ml/kg. Mean arterial pressure is recorded on a polygraph at 5, 10, 15, 20, and 30 min after initiation of drug administration. Seven to nine animals are used for each drug and dose level studied.

***α*-Adrenoreceptor blockade** can be determined in ganglion-blocked rats. Pressor responses to graded doses of phenylephrine injected intravenously are obtained before and 15 min after administration of test compounds. Sufficient concentrations of phenylephrine have to be given to ensure a rise in mean arterial blood pressure of 50 mmHg or more. Data obtained from five or six animals are averaged and resultant dose–response curves plotted. The dose of phenylephrine required to elicit a 50-mmHg increase in mean arterial blood pressure is interpolated from dose–response curves.

Standard data:

The following compounds are used as standards and, at the doses indicated, lower mean arterial blood pressure by about 50 mmHg:

- Cinnarizine 3.0 mg/kg, i.v.
- Hydralazine 1.0 mg/kg, i.v.
- Minoxidil 10.0 mg/kg, i.v.
- Saralasin 0.03 mg/kg, i.v.
- Molsidomine 0.1 mg/kg, i.v.

Evaluation

Mean values ± SEM are given for mean arterial blood pressure and heart rate. Changes of these parameters after drug administration are compared to control values obtained immediately before the application of the test compound. Statistical significance is assessed by means of the paired *t*-test.

Critical Assessment of the Method

A hypotensive response in this model appears to correlate more closely with antihypertensive activity in DOCA-salt hypertensive rats than does a vasodilator response in the perfused hind limb of anesthetized dogs and allows a distinction between central antihypertensive and vasodilators.

Modifications of the Method

Santajuliana et al. (1996) developed a standard ganglionic blockade protocol to assess neurogenic pressor activity in conscious rats. Rats were instrumented with arterial and venous catheters for measurement of arterial pressure and heart rate and for administration of three different ganglionic blockers (trimethaphan, hexamethonium, and chlorisondamine).

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Blood Pressure in Conscious Hypertensive Rats (Tail-Cuff Method)

Purpose and Rationale

Rats with spontaneous or experimentally induced hypertension are widely used for screening of potentially antihypertensive compounds. The indirect tail-cuff method allows the determination of systolic blood pressure according to the following principle: The cuff is quickly inflated to well above suspected systolic blood pressure; the pulse

will then be obliterated. Thereafter, pressure in the cuff is slowly released and, as the pressure falls below systolic blood pressure, the pulse will reappear. The method is analogous to sphygmomanometry in human and can be applied not only at the tail of awake rats but also in dogs and small primates. The indirect tail-cuff method is widely used to evaluate the influence of antihypertensive drugs in spontaneously and experimentally hypertensive rats.

Procedure

Male spontaneous hypertensive rats (Charles River) weighing 300–350 g or rats with experimentally induced hypertension are used.

Surgical Procedure to Induce Renal Hypertension

Male Sprague–Dawley rats weighing 80–100 g are anesthetized by intraperitoneal injection of 0.8 ml 4 % chloral hydrate solution. Both kidneys are exposed retroperitoneally. To induce renal hypertension, a silver clip (0.2 mm diameter, 4 mm length) is placed onto both renal arteries, the kidneys are reposed, and the wound is closed by suture.

Within 5–6 weeks, operated animals attain a renal hypertension with a systolic blood pressure (BPs) of 170–200 mmHg (mean normal physiological BPs for rats is 100 mmHg). Only animals with a BPs = 180 mmHg are used for the tests.

Test Procedure

The procedure is the same for spontaneously and experimentally hypertensive rats. Groups of six animals are used per dose. The control group receives saline only. To reduce spontaneous variations in blood pressure, animals are adjusted to the experimental cage by bringing them into the restraining cage which is enclosed in a 31–32 °C measuring chamber three to four times before the start of the experiment for a period of 30–60 min.

To measure blood pressure, a tubular inflatable cuff is placed around the base of the tail and a piezoelectric pulse detector is positioned distal to the cuff. The cuff is inflated to approximately 300 mmHg. As the pressure in the cuff is slowly released, the systolic pressure is detected and subsequently recorded on a polygraph.

The test substance is administered intraperitoneally or by gavage once per day over a period of 5 days. The usual screening dose of a new compound is 25 mg/kg. Blood pressure and heart rate measurements are taken at the following times:

Day 1: predose and 2 h postdrug
Day 3: predose and 2 h postdrug
Day 5: predose, 2 h postdrug, and 4 h postdrug

Between measurements, animals are returned to their home cages.

Standard compounds:

- Endralazine (3 mg/kg p.o.)
- Nifedipine (3 mg/kg p.o.)
- Urapidil (5 mg/kg p.o.)

Evaluation

Mean values in systolic blood pressure before and after drug administration and the duration of the effect are determined. Percent decrease in systolic blood pressure under drug treatment is calculated. Statistical significance is assessed by the Student's *t*-test.

Scores for % decrease in systolic blood pressure and for the duration of the effect are allotted.

Critical Assessment of the Method

The indirect tail-cuff method is being used in many laboratories with many modifications of the devices. Pfeffer et al. (1971) found a good correlation between values obtained with the indirect tail-cuff method and values measured directly with indwelling carotid arterial cannulae, whereas Buñag et al. (1971) reported a lack of correlation

between direct and indirect measurements of arterial pressure in unanesthetized rats, and Patten and Engen (1971) found difficulties to measure accurate systolic values at higher blood pressure. A good correlation between direct blood pressure data from the carotid artery in rats and readings with the tail-cuff method was found by Matsuda et al. (1987) who developed a six-channel automatic blood pressure measuring apparatus with a highly sensitive photoelectric sensor for the detection of tail arterial blood flow and a microcomputer system for automatic measurement of systolic blood pressure and heart rate and for data acquisition and processing.

Modifications of the Method

Details of the tail-cuff method in rats have been discussed by Stanton (1971).

Special equipment for measuring blood pressure in rats is commercially available (e.g., TSE GmbH, Bad Homburg, Germany).

Widdop and Li (1997) described a simple versatile method for measuring tail-cuff systolic blood pressure in conscious rats. A tail cuff consisting of a metal T-piece tube with latex rubber inside the tube is placed around the tail at the proximal end. A piezoelectric transducer (model MLT1010) is strapped to the ventral surface of the tail to record the pulse signal from the caudal artery and connected directly to a MacLab data-acquisition system (ADInstruments Pty Ltd.).

The tail-cuff method for measurement blood pressure has been adapted for dogs, monkeys (Wiester and Iltis 1976), and cats (Mahoney and Brody 1978).

Blood pressure can be measured from the hind leg of the rat using a leg cuff and a photoelectric cell situated at the dorsal surface of the foot (Kersten et al. 1947). When the leg is occluded, the foot swells and the amount of light striking the photocell is reduced. When the pressure in the cuff is released, the arterial blood flow is restored, the increase of foot volume is decreased, and the amount of light transversing the paw increases.

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Direct Measurement of Blood Pressure in Conscious Rats with Indwelling Catheter

Purpose and Rationale

The method first described by Weeks and Jones (1960) allows the direct measurement of arterial pressure in conscious rats eliminating the influence of anesthesia on cardiovascular regulation.

Procedure

Preparation of Cannulae

In order to prepare the cannulae, 7-cm- and 12-cm-long pieces are cut from PE-10 and PE-20 tubings, respectively. A stylet wire is inserted into the PE-10 tubing and the PE-20 tubing is also slipped over the stylet wire. The ends of the tubings are heated in a current of hot air and fused together. Ridges are made to anchor the cannula in the animal's tissue. In order to make a ridge, the stylet wire is left inside the cannula and the cannula is heated in a fine jet of hot air. When the polyethylene at the point of heating becomes soft, the cannula is pressed slightly and thus a ridge is formed. One ridge is formed at the PE-20 tubing, about 0.5 cm away from the junction with the PE-10 tubing, and 3 more ridges are formed on the PE-20 tubing at a distance of about 1 cm from each other, first one being situated about 3 cm away from the free end of the PE-20 tubing. The stylet wire is then removed from the cannula, and the PE-10 portion of the cannula near the junction with the PE-20 tubing is wound around a glass rod with a diameter of 4 mm. Two rounds are made. Then it is dipped in a boiling water bath for about 5 s. When taken out of the bath, the cannula retains its circles, forming a spring-like structure.

Implantation of Cannulae

Male Sprague–Dawley rats weighing about 300 g are used. The rat is anesthetized with 45 mg/kg pentobarbital i.p. The area of the neck and the abdomen are shaved and cleaned with 70 % alcohol. The viscera are exposed through a midline abdominal incision. A segment of the abdominal aorta is exposed just above the bifurcation. A trocar is passed through the psoas muscles adjacent to this segment of the aorta, through the muscles of the back and under the skin until it emerges from the skin of the neck. Then the cannula is inserted into the trocar and the trocar is withdrawn from the body. The end of the cannula thus comes out from the neck, being anchored by silk sutures to the neck skin and to the psoas muscle. The cannula is filled with heparin solution and the end which is projecting out from the neck skin is blocked with a tight fitting stainless steel needle. Then the other end of the cannula is implanted into the aorta. The aorta is wiped with a cotton-tipped applicator stick above the bifurcation, occluded above this segment, and punctured with a bent 27-gauge hypodermic needle. The tip of the PE-10 catheter is inserted through the needle and advanced up the aorta. The intestines are replaced and the wound sutured. The rats are allowed to recover for one week.

Measurement of Blood Pressure

The occluding stainless steel needle is removed and the cannula flushed with diluted heparin solution. The rat is placed in a small cage to restrict its movements, even so it is free to move. The cannula is connected to a Statham P23 DB pressure transducer, and blood pressure is recorded on a polygraph. Test drugs or standards are administered either subcutaneously or orally. Recordings are taken before and after administration of drug over a period of 1 h.

Evaluation

Changes of blood pressure are measured for degree and duration. Five rats are used for each dose and

compound. The maximal changes of each group are averaged and compared with the standard.

Critical Assessment of the Method

Direct measurement of arterial blood pressure in unanesthetized rats originally introduced by Weeks and Jones (1960) has become a valuable and widely used tool in cardiovascular research.

Modifications of the Method

A detailed description of a slightly modified Week's method has been given by Stanton (1971).

Improvements of the method for continuous direct recording of arterial blood pressure and heart rate in rats have been described by Buñag et al. (1971), Laffan et al. (1972), Buckingham (1976), Garthoff and Towart (1981), and Garthoff (1983). A detailed description of permanent cannulation of the iliolumbar artery was given by Remie et al. (1990).

Wixson et al. (1987) described a technique for chronic catheterization of the carotid artery in the rat. Prepared cannulae are commercially available (IRC Life Science, Woodland Hills, CA).

A newer modification uses the access to the aorta via the common carotid artery (Linz et al. 1992). Rats are prepared under thiopental anesthesia with arterial PE-50 lines (Intramedic from Clay Adams, USA). The lines are introduced into the ascending aorta via the right carotid artery for direct measurement of arterial blood pressure and into the jugular vein for i.v. application of test compounds. Both lines, filled with saline containing heparin, are surfaced on the neck. The animals are allowed to recover for at least 2 days. Blood pressure is monitored through Statham R P23DB transducers connected to a recording device. During measurements, the lines are kept open with countercurrent saline infusion at a rate of 1 ml/h.

Bao et al. (1991) placed one catheter via the right femoral artery in the abdominal aorta in rats for recording mean arterial pressure and two additional catheters via the left carotid artery into the descending aorta for application of bradykinin and bradykinin antagonists.

Arterial pressure was recorded in unanesthetized rats after induction of severe hypertension by complete ligation of the aorta between the origin of the renal arteries by Sweet and Columbo (1979).

Hilditch et al. (1978) described a device for the direct recording of blood pressure in conscious dogs.

Akrawi and Wiedlund (1987) described a method for chronic portal vein infusion in unrestrained rats. Hepatic drug metabolism can be studied by infusion into the portal vein and blood collection from the femoral vein.

Robineau (1988) described a method for recording electrocardiograms in conscious, unrestrained rats. Electrodes were implanted subcutaneously and a socket connector was sutured on the head of the animal. A flexible cord leading to a swivel collector was linked to an ECG amplifier.

Kurowski et al. (1991) reported on an improved method to implant, maintain, and protect arterial and venous catheters in conscious rats for extended periods of time.

Schenk et al. (1992) measured cardiac left ventricular pressure in conscious rats using a fluid-filled catheter.

Tsui et al. (1991) recommended a reliable technique for chronic carotid arterial catheterization in the rat.

Hagmüller et al. (1992) described a tail-artery cannulation method for the study of blood parameters in freely moving rats.

Liebmann et al. (1995) described an in vivo long-term perfusion system which is based on 195 automated, computer-controlled high-frequency heparin (10 U/ml) flushing of a cannula inserted into the tail artery of freely moving rats.

Santajuliana et al. (1996) used conscious rats instrumented with arterial and venous catheters to assess neurogenic pressor activity after administration of ganglionic blockers.

Rezek and Havlicek (1975) described simple cannula systems for the infusion of experimental substances in chronic, unrestrained animals. A cannula with a removable cap is used for

infusions into various parts of the digestive tract. Intravenous infusions can be performed through a closed system cannula which avoids a possible introduction of air into the circulation.

Kimura et al. (1988) described a method for chronic portal venous, aortic, and gastric cannulation to determine portal venous and aortic glucose and lactate levels in conscious rats.

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Cannulation Techniques in Rodents

Purpose and Rationale

Cardiovascular pharmacology requires special techniques for catheterization and permanent cannulation of vessels. A few methods are described below.

A comprehensive literature survey on methods for vascular access and collection of body fluids from the laboratory rat was written by Cocchetto and Bjornsson (1983).

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Permanent Cannulation of the Jugular Vein in Rats

Purpose and Rationale

Permanent cannulation of the jugular vein in rats in combination with a head attachment apparatus allowing easy connection of cannulae was first introduced by Steffens (1969). Modifications were described by Brown and Hedge (1972), by Nicolaidis et al. (1974), and by Dons and Havlik (1986). A detailed description was given by Remie et al. (1990).

Procedure

Rats are anesthetized with N_2O_2/O_2/halothane. The shaven neck of the animal on the right side is disinfected with chlorhexidine solution. The incision is made just above the right clavicle. Connective and adipose tissue are pushed aside with blunt forceps and the jugular vein is exposed. The external jugular vein is followed and the division into the maxillary vein, the facial, and the linguofacial vein identified. The largest vein is chosen and mobilized for a distance of about 5 mm. Small artery forceps are used to clamp the vessel. The vein is then ligated rostral to the clamp with 6–0 silk, and a second ligature is put loosely around the vessel, but not tightened. Using iridectomy scissors, a V-shaped hole is cut in the vein 2 mm rostral from the bifurcation. Prior to its insertion into the vessel, a sterile cannula is connected to a 1-ml syringe filled with a heparinized saline solution. The vessel is dilated by means of a sharp pointed jeweler's forceps, the cannula slit between the legs of the forceps and gently pushed into the vessel until the tip is at the level of the right atrium. Then the forceps is removed, the caudal ligature gently tied, and the rostral ligature used to anchor the cannula to the vessel. The cannula is tunneled to emerge at the top of the head. While the skin in the neck is held firmly, the artery forceps is inserted subcutaneously in caudal direction over a distance of about 3 cm, then turned anticlockwise in the direction of the incision in the neck. The cannula is grasped with the forceps. Then the forceps is pulled back until the cannula emerges at the crown of the head and closed by a small microvascular clamp. The cannula is slid over the short end of a 20G stainless steel needle bent to a 90° angle. The catheter is flushed with saline and filled with polyethylene/heparin solution. The long end of the L-shaped stainless steel adapter is closed with a piece of heat-sealed PE tubing, and the wounds are closed with sutures.

Modifications of the Method

Hutchaleelaha et al. (1997) described a simple apparatus for serial blood sampling from the external jugular vein which permits simultaneous measurement of locomotor activity in freely moving rats.

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Permanent Cannulation of the Renal Vein in Rats

Purpose and Rationale

A detailed description for permanent cannulation of the renal vein in rats was given by Remie et al. (1990).

Procedure

Rats are anesthetized with N_2O_2/O_2/halothane. After opening the abdominal wall, the intestines are lifted out and laid next to the animal on the right side (as viewed by the surgeon) on gauze moistened with warm saline solution. This provides an excellent view to the vena cava. At its confluence with the vena cava, the right renal vein is stripped of its adipose tissue and the peritoneum is opened. Using small anatomical forceps, the peritoneum is detached from the vena cava by making small spreading movements with the forceps just beneath the peritoneum. Subsequently, the vena cava and the renal vein are mobilized for approximately 1.5 cm, to allow for clamping of the vessel. A four or five fine-stitch purse string is placed in the vessel at the confluence of the vena cava and the right renal vein. Using a Barraquer needle holder and a cotton-wool stick, the 7–0 silk suture, armed with a BV-1 needle, is guided through the vessel. After each stitch, any bleeding has to be immediately arrested by applying light pressure using a cotton-wool stick. Having completed the suture, a single knot is made with the drawstrings. Three microvascular clips are then placed on the vena cava and the renal vein: first the proximal clip on the vena cava, followed by the clip on the renal vein, and finally the distal vena cava clip. A small aperture is cut immediately inside the purse-string suture using iridectomy scissors and jeweler's forceps. The cannula, which is filled with a heparinized saline solution, is pushed into the opening as far as possible. The purse-string suture is pulled taut and the clip of the renal vein removed, while pushing the cannula further. The proximal clip on the vena cava is now removed as quickly as possible. The patency of the cannula is checked and the drawstrings of the purse-string suture are used to anchor the cannula. The cannula is laid kink-free in the abdominal cavity and sutured to the internal abdominal cavity near the xiphoid cartilage. The abdomen is closed in two layers and the cannula tunneled to the top of the head.

The cannula together with a L-shaped adapter is fixed to the skull.

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Permanent Cannulation of the Portal Vein in Rats

Purpose and Rationale

Several techniques have been described for cannulation of the portal vein in rats (Hyun et al. 1967; Pelzmann and Havemeyer 1971; Suzuki et al. 1973; Sable-Amplis and Abadie 1975; Helman et al. 1984). A detailed description for permanent cannulation of the portal vein in rats was given by Remie et al. (1990). After additional application of platinum electrodes around the portal vein in close proximity to the catheter tip, this model can also be used to study the presynaptic regulation of neurotransmitter release from nonadrenergic nerve terminals (Remie and Zaagsma 1986; Remie et al. 1988, 1989).

Procedure

Rats are anesthetized with N_2O_2/O_2/halothane. After opening the abdominal wall, the intestines are lifted out and laid next to the animal on the right side (as viewed by the surgeon) on gauze moistened with warm saline solution. Using a microneedle holder and a cotton-wool stick, a four or five fine-stitch purse-string suture (7–0 silk suture armed with a BV-1 needle) is placed in the wall of the portal vein at the side opposite the gastroduodenal vein. The diameter of the purse string should be about 1 mm. After the suture has been completed, a single knot is made with the drawstrings. The portal vein is clamped with a small curved hemostatic bulldog clamp. Using iridectomy scissors and a pair of jeweler's forceps, the center of the purse string is cut; a cannula filled with heparinized saline is inserted into the vessel and pushed upwards. The purse string is gently tightened taking care not to obstruct the cannula. The drawstrings of the suture are used to anchor the cannula. The cannula is laid kink-free in the abdominal cavity and sutured to the internal abdominal cavity near the xiphoid cartilage. The abdomen is closed in two layers and the cannula tunneled to the top of the head. The cannula together with a L-shaped adapter is fixed to the skull.

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Permanent Cannulation of the Thoracic Duct in Rats

Purpose and Rationale

Collection of lymph is rather difficult and has performed mainly in dogs (Biedl and Offer 1907; Gryaznova 1962, 1963; Vogel 1963). Some techniques have been described for the rat (Bollman et al. 1948; Girardet 1975). Remie et al. (1990) did not obstruct the duct by placing a purse-string suture in the wall of the duct, by which the cannula is secured. The animal's lymph can be collected during the experiment, and after refilling the cannula, the lymph flow remains undisturbed.

Procedure

Rats are anesthetized with N_2O_2/O_2/halothane. After opening the abdominal cavity, the intestines are placed in gauze moistened with warm saline and laid to the left of the animal. The suprarenal abdominal artery is located and mobilized by gently tearing the connective tissue. Using blunt dissection technique, the thoracic duct is mobilized along the dorsolateral surface of the aorta. A small three to four fine-stitch purse-string suture is placed in the wall of the duct, using a 9–0 Ethilon suture. A hole is cut inside the purse string with a very fine pair of scissors, while holding the wall with angled jeweler's forceps. The cannula is filled with heparinized saline solution and is inserted into the duct using anatomical forceps. After the tip of the cannula has been inserted into the thoracic duct, the curved forceps are removed and the total tip is pushed into the duct. The ligature is then closed and some lymph will flow into the cannula. The cannula is secured within the abdominal cavity by attaching it to the abdominal muscle near the xiphoid cartilage with a 7–0 silk suture. Following the closure of the abdominal wall and the tunneling of the cannula to the crown of the head, an L-shaped adapter is placed on the cannula, filled with PVP solution and closed with a heat-sealed polyethylene cap.

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Portacaval Anastomosis in Rats

Purpose and Rationale

In 1877 the Russian surgeon Eck reported the achievement of successful portacaval shunts in dogs. Lee and Fischer (1961), Funovics et al. (1975), and de Boer et al. (1986) described

portacaval shunt in the rat. A detailed description of surgery for portacaval anastomosis in rats was given by van Dongen et al. (1990).

Procedure

Rats are anesthetized with N_2O_2/O_2 in combination. After opening the abdominal wall, the intestines are placed left to the animal on gauze moistened with warm saline solution. Proximally and distally to the animal's right renal vein, the vena cava is then stripped of its adipose and connective tissue, and the retroperitoneal cavity is opened. Using anatomical forceps, the peritoneum is dissected from the vena cava by making small spreading movements with the forceps just above the vena cava. The portal vein is pulled slightly to the left using straight anatomical forceps and freed from the hepatic artery and the gastroduodenal artery with curved anatomical forceps. Rostral to the celiac artery, the abdominal artery which is covered with peritoneum is freed from its lateral muscle bed over a length of approximately 5 mm providing enough space for placing a small bulldog clamp at a later stage of the operation. Without occlusion, a six fine-stitch purse string is placed in the wall of the vena cava close to its confluence with the right renal vein. Using a Barraquer needle holder and a cotton-wool stick, the 7–0 silk suture armed with a BV-1 needle is guided through the vessel. After each stitch, bleeding has to be arrested immediately, by applying light pressure on the area, again using the cotton-wool stick. After the suture has been completed, a single knot is made with the drawstrings. The drawstrings should come together at the rostral part of the purse string. A bulldog clamp, modified to resemble a Satinsky vascular clamp, is then placed on the vena cava.

Before clamping the abdominal aorta rostral to the celiac artery with a small bulldog clamp, a ligature (7–0 silk) is placed around the portal vein as close as possible to the hilus of the liver. Subsequently, the clamp is placed on the aorta and the ligature tightened. A Heifetz clip is then placed transversely onto the portal vein at its confluence with the gastroduodenal vein. The portal vein is cut just distally from the ligature. A prepared button is slipped over the left-hand straight small anatomical forceps, while the right-hand forceps are used to pass the portal vein to the left-hand anatomical forceps. The vein is then grasped and pulled through the button. Subsequently, the button is pushed as close as possible to the Heifetz clip and clamped to the clip using a Pilling bulldog clamp.

Using small straight and curved anatomical forceps, the portal vein is reversed around the button and fixed with a previously prepared 7–0 silk suture. The Pilling bulldog clamp is then removed and replaced at the end of the Satinsky clamp for reasons of stability. The vena cava is then somewhat elevated, bringing it into closer contact with the portal vein button.

A longitudinal cut is made in the purse-string suture using iridectomy scissors and jeweler's forceps. One drawstring of the suture is clamped with a small hemostat and put under slight tension in a rostral direction. The button manipulated by its grip is pushed into the vena cava. The purse string is tightened with the left hand while the right hand still holds the button in position. The button is released and two additional knots tied. The Satinsky clamp is removed first followed by the Heifetz clamp and the bulldog clamp on the aorta. After replacing the intestines, the abdominal wall is closed in two layers.

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Cardiovascular Analysis in Anesthetized Mice

Purpose and Rationale

To fully utilize the potential of mouse models with specific gene mutations, it is necessary to study the functional consequences of genetic manipulations in fully intact mice. Lorenz and Robbins (1997) developed and validated a methodology to study cardiovascular parameters in closed-chest mice.

Procedure

Adult mice of either sex weighing 25–35 g are anesthetized by intraperitoneal injection of 50 mg/kg ketamine and 100 mg/kg thiobutabarbital. After the mice are placed on a thermally controlled surgical table with body temperature continually monitored via a rectal probe, a tracheotomy is performed with a short length (<1 cm) of PE-90 tubing. The right femoral artery is then cannulated with polyethylene tubing which is pulled over a flame to a small diameter (~0.4 mm OD). The catheter is advanced ~1 cm, near the level of the aorta, and connected directly to a low-compliance COBE CDXIII fixed-dome pressure transducer for the measurement of arterial blood pressure. The right femoral vein is then cannulated with the same type of small-diameter tubing and connected to a microinjection pump for the infusion of experimental drugs. To assess myocardial performance, the right carotid artery is cannulated with a 2 F Millar MIKROTIP transducer (Model SPR-407, Millar Instruments, Houston TX). This high-fidelity transducer, which has a tip diameter of ~0.67 mm, has a reported frequency response that is flat up to 10,000 Hz and therefore can be used to accurately monitor the high frequency of the mouse ventricular pulse pressure. During continual monitoring of the blood pressure wave to ascertain the anatomic position of the catheter, the tip of the transducer is carefully advanced through the ascending aorta and into the left ventricle. When the stable waveform of the ventricular pressure profile is achieved, the transducer is anchored in place with 7–0 silk sutures. After completion of the surgery, all wounds are closed with cyanoacrylate to minimize evaporative loss of fluid, and the animals are allowed to stabilize for 30–45 min.

Evaluation

Blood pressure signals from the COBE transducer and from the Millar transducer are amplified, and the output is recorded and analyzed with a MacLab 4/s data-acquisition system connected to a Macintosh 7100/80 computer which allows the calculation of the following parameters:

dP/dt first derivative of the ventricular pressure wave
MAP mean arterial pressure
HR heart rate
LVP systolic and diastolic left ventricular pressure
LVEDP left ventricular end-diastolic pressure

Further indices of ventricular performance can be calculated from d*P*/d*t*.

Modifications of the Method

Champion et al. (2000) described a **right-heart catheterization technique** for in vivo measurement of vascular responses in lungs of intact mice. CD1 mice weighing 25–38 g were anesthetized with thiopentobarbital (85–95 mg/kg i.p.) and ketamine (3 mg/kg i.p.) and were strapped in

supine position to a thermoregulated fluoroscopic table. The trachea was cannulated and the animals breathed with room air enriched with 95 % O_2/5 % CO_2. A femoral artery was cannulated for the measurement of systemic arterial pressure. Heart rate was electronically monitored from the systolic pressure pulses with a tachometer (Grass model 7P44A). The left jugular vein was cannulated for the administration of agonists and antagonists.

For measuring pulmonary arterial pressure, a special single lumen catheter was constructed. The catheter was 145 mm in length and 0.25 mm in outer diameter, with a specially curved tip to facilitate passage through the right heart, main pulmonary artery, and the left or right pulmonary artery. Before the catheter was introduced, the catheter curve was initially straightened with a 0.010-in. straight angioplastic guide wire to facilitate passage from the right jugular vein into the right atrium at the tricuspid valve under fluoroscopic guidance. As the straight wire was removed, the natural curve facilitated entry of the catheter into the right ventricle. A 0.010-in. soft-tip coronary artery guide wire was then inserted, and the catheter was passed over the guide wire into the main pulmonary artery under fluoroscopic guidance. Pressure in the main pulmonary artery was measured with a pressure transducer, and mean pulmonary artery pressure was derived electronically and recorded continuously.

Cardiac output was measured by the thermodilution technique. A known volume (20 µl plus catheter dead space) of 0.9 % NaCl solution at 23 °C was injected into the right atrium, and changes in blood temperature were measured at the root of the aorta. A cardiac output computer equipped with a small animal interface was used. The thermistor microprobe was inserted into the right carotid artery and advanced to the aortic arch, where changes in aortic blood temperature were measured. A catheter placed in the right jugular vein was advanced to the right atrium or main pulmonary artery for rapid bolus injection of saline. The saline solution was injected with a constant-rate syringe to ensure rapid and repeatable injection of the saline indicator solution. Thermodilution curves were recorded on a chart recorder and pulmonary and systemic blood pressure monitored continuously. Catheter placement was verified by postmortem examination.

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Blood Pressure in Anesthetized Cats

Purpose and Rationale

Cats are the most sensitive species of cardiovascular regulation. They were used extensively for cardiovascular screening. Recently, experiments in dogs are preferred since this species can be bred more easily in homogeneous strains.

Procedure

Adult cats of either sex weighing 2.5–4 kg are anesthetized by intraperitoneal injection of 35 mg/kg sodium pentobarbital. Tracheotomy is performed and a tracheal cannula is inserted so that the cat can be mechanically ventilated with room air. A femoral artery and two femoral veins are cannulated for measurement of arterial blood pressure and systemic administration of drugs. The arterial cannula is connected to a Statham model P23Gb transducer. All recordings are made on a polygraph. Heart rate is determined with a Beckman Cardiotach connected to a voltage/pressure-pulse coupler. Rectal temperature is monitored and maintained between 37 °C and 38 °C with a heating pad.

The following drugs are injected i.v. as challenges:

- Epinephrine 0.1, 0.3, 0.5 µg/kg
- Norepinephrine 0.1, 0.3, 0.5 µg/kg
- Isoproterenol 0.1, 0.2, 0.4 µg/kg
- Carbachol 0.1, 0.2, 0.5 µg/kg

At least 5 min are allowed between challenge doses to permit the measured parameters to return to baseline.

Test drugs are injected at various doses followed by injections of the challenging drugs.

Evaluation

Dose–response curves of challenging drugs are established before and after injections of the test drugs.

Critical Assessment of the Method

Blood pressure experiments in anesthetized cats are very valuable as screening techniques for cardiovascular agents. Moreover, potentiation of norepinephrine response has been used as screening procedure for antidepressants with norepinephrine uptake inhibiting activity.

Modifications of the Method

Sander (1965) investigated the vasoconstrictor and vasodilator effects of procaine in spinal cats. The animals were anesthetized with ether and ventilated with a positive pressure pump via a tracheal cannula. The spinal cord was then cut between the second and third vertebrae, and ether administration stopped. The remaining portion of the spinal cord above the transection was destroyed by passing a curette through the spinal canal.

Yardley et al. (1989) studied cardiovascular parameters in spinal cats. The animals were anesthetized with 80 mg/kg intravenously administered α-chloralose. The spinal cord was transected or crushed at the first cervical segment after tetracaine hydrochloride (0.125 mg in 0.1 ml) had been injected into this region of the cord. Systemic blood pressure was supported at a level sufficient to maintain constricted pupils (mean value 45 ± 5 mmHg) by volume expansion with blood from a donor (10–20 ml) or an infusion of dextran.

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Cardiovascular Drug Challenging Experiments in Anesthetized Dogs

Purpose and Rationale

Sympathomimetic and cholinomimetic compounds as well as angiotensin II and carotid occlusion exert characteristic responses in blood pressure of anesthetized dogs. Antagonism or potentiation of these responses allows to characterize the cardiovascular activity of a new compound.

Procedure

Adult Beagle dogs of either sex weighing between 8 and 15 kg are anesthetized with 15 mg/kg sodium thiopental, 200 mg/kg sodium barbital, and 75 mg/kg sodium pentobarbital. Additional doses of sodium pentobarbital are given as needed. The dogs are intubated with a cuffed endotracheal tube and placed on a Harvard respirator (20 ml/kg, 10–15 cycles/min). A femoral vein and artery are cannulated using polyethylene tubing for drug administration and determination of arterial blood pressure, respectively. The animals are bilaterally vagotomized.

The arterial cannula is connected to a Statham model P23Gb transducer. All recordings are made on a polygraph. Heart rate is determined with a Beckman Cardiotach connected to a voltage/pressure-pulse coupler.

Drug challenges: One of the following combinations of drugs is administered i.v. to the dogs. The challenges are given in a fixed order: at least twice prior to test-drug administration to insure consistent responses and again starting 15 min posttest drug. Epinephrine and norepinephrine (1 μg/kg), isoproterenol (0.25 μg/kg), carbachol (0.25 μg/kg), tyramine (100 μg/kg), bilateral carotid occlusion (45 s), phenylephrine (10 μg/kg), isoproterenol (0.25 μg/kg), angiotensin II (0.2 μg/kg), and carbachol (0.25 μg/kg). At least 5 min are allowed between challenge doses to permit the measured parameters to return to baseline. Challenge drug doses are sometimes varied to keep the mean arterial pressure within the following limits: epinephrine (+30 to +60 mmHg), norepinephrine (−30 to +70 mmHg), tyramine (+30 to +70 mmHg), isoproterenol (−30 to −50 mmHg), carbachol (−30 to −50 mmHg), phenylephrine (−30 to +70 mmHg), angiotensin II (+30 to +50 mmHg), and bilateral carotid occlusion (+30 to +70 mmHg).

Evaluation

The recordings are studied to detect any changes in the arterial pressure response to the challenge drug before and after test-drug administration and to observe any changes in blood pressure and heart rate. Results are expressed as the percentage change from the predrug response.

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Hemodynamic Analysis in Anesthetized Dogs

Purpose and Rationale

The hemodynamic effects of compounds supposed to affect the cardiovascular system are evaluated by measuring preload and afterload

of the heart, contractility, heart rate, cardiac output, and peripheral or coronary flow. To measure these cardiovascular parameters accurately, the use of larger animals such as dogs or pigs is necessary.

This experimental model allows the classification of test drugs according to their action as having:

- Positive inotropic effects
- Negative inotropic effects (Ca^{2+}-antagonist, antiarrhythmic?)
- Hypertensive effects
- Hypotensive effects
- Coronary-dilating effects
- β-Blocking effects
- α-Blocking effects
- Antianginal effects
- Peripheral vasodilating effects

Procedure

Male or female inbred Beagle or Labrador–Harrier dogs weighing between 15 and 25 kg are used. They are anesthetized with a bolus injection of 35–40 mg/kg pentobarbital and continued with an infusion of 4–6 mg/kg/h. A catheter is placed into the cephalic vein for intravenous injections. Another catheter is placed into the duodenum for enteral administration. Respiration is maintained with room air through a tracheal tube using a positive pressure respirator, e.g., Bird Mark 7 respirator. Blood gas analyses are performed at regular time intervals. Oxygen is supplied via the respirator as needed.

Preparation for Hemodynamic Measurements

Blood pressure is recorded through a cannula inserted into the left femoral artery and connected to a Statham pressure transducer (Statham P23 DB).

For determination of LVP, a Millar microtip catheter (type PC 350) is inserted via the left common carotid artery into the left ventricle. LVEDP is measured on a high-sensitivity scale. From the pressure curve, dp/dt_{max} is differentiated and heart rate is counted. The LVP signal also triggers a cardiotachometer.

Cardiac output, pulmonary artery pressure (PAP), and stroke volume are measured by a thermodilution technique using a cardiac output computer (Gould/Statham SP 1245) and a balloon-tip triple-lumen catheter (Gould SP 5105, 5F) with the thermistor positioned in the pulmonary artery via the jugular vein.

Myocardial oxygen consumption (MVO_2) is calculated as pressure-work index according to Rooke and Feigl (1982).

Femoral blood flow and coronary flow are measured with electromagnetic flow probes attached to the femoral artery and the circumflex branch of the left coronary artery (LCX), respectively.

Experimental Course

When stable hemodynamic conditions and blood gas values of $pO_2 > 100$ mmHg and $pCO_2 < 35$ mmHg are achieved for at least 20 min (control values), the test substance is administered through a catheter inserted into a cephalic vein in doses of 0.1, 0.3, 1.0, and 3.0 mg/kg or into the duodenum in doses of 0.3, 1.0, 3.0, and 10.0 mg/kg.

All parameters are recorded continuously during the whole experiment.

Characteristics

- Blood pressure
  - Systolic, BPs
  - Diastolic, BPd
- Left ventricular pressure, LVP
- Left ventricular end-diastolic pressure, LVEDP
- Maximal rate of pressure rise, dp/dt max
- Heart rate, HR
- Peripheral blood flow in A. femoralis, PF
- Blood pressure in A. pulmonalis, PAP
- Coronary flow, CF
- Cardiac output, CO
- Stroke volume, SV
- Total peripheral resistance, TPR
- Left ventricular stroke work, LVSW
- Left ventricular minute work, LVMW
- Left ventricular myocardial oxygen consumption, MVO_2

Calculation of Results and Evaluation

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

- Stroke volume [ml/beat],

$$SV = \frac{CO}{HR}$$

- Total peripheral resistance [dyns/cm^5],

$$TPR = \frac{BPm}{CO} \times 79.9$$

- Left ventricle stroke work [J/beat],

$$LVSW = (BPm - LVEDP) \times SV \times 0.333 \times 10^{-3}$$

- Left ventricular minute work [J/min],

$$LVMW = LVSW \times HR$$

- Left ventricular myocardial oxygen consumption [ml O_2/min/100 g],

$$MVO_2 = K_1(BPs \times HR) + K_2 \times \frac{(0.8BPs + 0.2BPd) \times HR + SV}{BW} + 1.43$$

$K_1 = 4.08 \times 10^{-4}$
$K_2 = 3.25.08 \times 10^{-4}$
BPs = systolic blood pressure [mmHg]
BPd = diastolic blood pressure [mmHg]
BPm = mean blood pressure [mmHg]
HR = heart rate [beats/min]
CO = cardiac output [ml/min]
SV = stroke volume [ml/beat]
$LVEDP$ = left ventricular end-diastolic pressure [mmHg]
BW = body weight [kg]

Changes in parameters measured after drug administration are compared to control values obtained during the 20-min predrug period.

Results are presented as mean ± SEM with $n > 3$.

Statistical significance is assessed by means of the paired *t*-test.

Modifications of the Method

The effect of drugs on the carotid artery occlusion effect can be studied in anesthetized dogs. The occlusion of right and left common carotid arteries is performed by squeezing them between a polyethylene tubing and a twine which is passed inside the tubing and around the carotid artery. An occlusion of the carotid arteries for 30 s causes an increase of systolic blood pressure by 40–50 mmHg. Inhibition of this effect by drugs is tested.

Studies in anesthetized dogs can be used to determine the influence of cardiotonic drugs on propranolol-induced cardiac insufficiency (Rajagopalan et al. 1993).

Instead of dogs, **pigs (German landrace)** weighing between 20 and 35 kg can be used. They are pretreated with ketamine 500 mg/5 ml i.m., metomidate hydrochloride 200 mg/4 ml i.p., and xylazine 60 mg/3 ml i.m. and anesthetized with 15–20 mg/kg sodium pentobarbital, followed by continuous infusion of 12 mg/kg/h. The parameters are evaluated similarly to the experiments in dogs.

Measurement of cardiac output by the thermodilution method in **rats** was described by Richardson et al. (1962) and Müller and Mannesmann (1981).

Thermodilution methods were used by Rosas et al. (1964) in anesthetized rats and by Carbonell et al. (1985) and by Salyers et al. (1988) in conscious rats to determine hemodynamic parameters.

Oxygen pressure, carbon dioxide pressure, and pH in coronary venous and common carotid arterial blood of anesthetized dogs have been measured using a blood gas analyzer (Aisaka et al. 1988).

Acute ischemic left ventricular failure can be induced in anesthetized dogs by repeated injections of plastic microspheres into the left coronary artery (Smiseth and Mjøs 1982; Sweet et al. 1984; Schölkens et al. 1986).

A coronary catheter was introduced through the right femoral artery and advanced under fluoroscopy to the left coronary ostium, guided by injection of small amounts of contrast medium. After reaching baseline values, acute left ventricular failure was induced by subsequent intracoronary injections of plastic microspheres (52.9- ± 2.48-μm nonradioactive tracer microspheres). The microspheres were suspended in saline with a drop of Tween 80 and sonified before use, 1 mg microspheres/1 ml saline corresponding to approximately 12,000 microspheres (13–16 injections of microspheres or 3.4–5.0 mg/kg). Microspheres were injected every 5 min for 70–90 min. Each microsphere injection effected an immediate and stepwise increase in LVEDP. With this procedure, LVEDP can be increased to a desired level in a very controlled manner. In the 30 min following embolization, LVEDP continued to increase by approximately 5 mmHg. Animal with arrhythmias had to be excluded from the study. Thirty min after the end of embolization, when hemodynamic parameters had stabilized, drug administrations were started.

Valdes-Cruz et al. (1984) developed an **open-chest preparation in dogs** to validate the accuracy of a two-dimensional Doppler echocardiographic method for estimating pressure drops across discrete stenotic obstructions.

In order to assess the potential of a single-breath technique (using Freon 22) as an effective way to estimate cardiac output noninvasively, Franks et al. (1990) measured simultaneously with the single-breath technique the aortic flow using an electromagnetic flowmeter in anesthetized dogs.

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Hemodynamic Measurements in Conscious Dogs

Purpose and Rationale

The potency of a cardiovascular drug depends on the direct effects at the cellular level and on the response of the cardiovascular control mechanisms. The latter are often markedly influenced by anesthesia. The chronically instrumented conscious dog with renal hypertension is therefore a more realistic test model to evaluate the effects of antihypertensive, antianginal, and cardiotonic compounds. The test is used to evaluate hemodynamic drug effects in conscious dogs, an experimental model with chronic arterial and ventricular catheterization and renal artery constriction.

Procedure

Male or female Labrador–Harrier dogs weighing 15–25 kg are used. They are anesthetized with 1 mg/kg xylazine i.m., followed by 1 mg/kg xylazine i.v. and 18 mg/kg sodium pentobarbital i.v. For chronic instrumentation and induction of renal hypertension, fluid-filled catheters are implanted into the abdominal aorta and into the left ventricle. The catheters are tunneled subcutaneously and exteriorized on the nape of the neck dorsally. Renal hypertension is induced by placing silastic constrictors around both renal arteries. Hemodynamic measurements are performed after a two-week recovery period or later.

To familiarize the dogs to the test surroundings, they are brought into the laboratory two to three times before the start of the study. Thus, drug testing is possible without sedation. During the experiment, the animal rests quietly on a laboratory table.

Experimental Protocol

Hemodynamic measurements are performed by connecting the two implanted catheters to Statham pressure transducers. Pressure signals, electronically differentiated LVP dp/dt max and heart rate are recorded with a polygraph.

After reaching stable hemodynamic conditions for at least 20 min (control baseline values), the test compound is administered either orally in a gelatin capsule or by intravenous injection into the cephalic vein.

Hemodynamic parameters are recorded continuously starting 30 min before to 120 min after drug administration and thereafter at 1-h intervals until 6 h after dosage.

Evaluation

The following parameters are monitored:

- Systolic blood pressure [mmHg]
- Diastolic blood pressure [mmHg]
- Left ventricular end-diastolic blood pressure, LVEDP [mmHg]
- Left ventricular pressure at dp/dt max [mmHg/s]
- Heart rate [beats/min]

Mean values ± SEM are calculated with $n > 3$ as differences to predrug control values.

Modifications of the Method

Mann et al. (1987) described a simple procedure for direct blood pressure measurement in conscious dogs using the Vascular Access Port™, consisting of a 33 × 13-mm reservoir body affixed to a silicone rubber catheter.

Müller-Schweinitzer (1984) described a method for the assessment of vasoconstrictor agents by recording venous compliance in the conscious dog. Changes in the diameter of the canine saphenous vein, produced by inflation to 45 mmHg of a sphygmomanometer cuff placed on the upper hind leg, were recorded.

Hintze and Vatner (1983) compared the effects of nifedipine and nitroglycerin in conscious dogs, instrumented for instantaneous and continuous measurements of coronary arterial and left ventricular diameters with an ultrasonic dimension gauge, arterial and left ventricular pressure with implanted miniature gauges, and coronary blood flow with an electromagnetic flowmeter or a Doppler ultrasonic flowmeter.

Shimshak et al. (1986) studied the recovery of regional myocardial contractile function after a 10-min coronary artery occlusion in chronically instrumented conscious dogs.

Wright et al. (1987) described a minimally invasive technique which allows assessment of histamine H_1-receptor antagonist activity in conscious dogs based on the inhibition of tachycardia caused by intravenous administration of the H_1-receptor agonist, 2-pyridylethylamine.

Hashimoto et al. (1991) studied the coronary effects of nicorandil in comparison with nitroglycerin in chronic conscious dogs instrumented with ultrasonic crystals and electromagnetic flowmeters in the circumflex coronary artery.

Hartman and Warltier (1990) described a model of multivessel coronary artery disease using conscious, chronically instrumented dogs. A hydraulic occluder and an ameroid constrictor were implanted around the left anterior descending and the left circumflex coronary arteries. Pairs of piezoelectric crystals were implanted within the subendocardium of the left anterior descending and the left circumflex coronary artery 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.

Hof et al. (1990) used the Doppler method for measuring cardiac output in **conscious rabbits**.

Grohs et al. (1993) simultaneously assessed cardiac output with pulsed Doppler and electromagnetic flowmeters during cardiac stimulation.

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Hemodynamic Studies in Monkeys

Purpose and Rationale

Prior to studies in human beings, studies of cardiovascular effects of new drugs in monkeys are necessary in some instances.

Procedure

Rhesus monkeys of either sex weighing between 5 and 8 kg are anesthetized with 20 mg/kg ketamine hydrochloride followed by 50 mg/kg pentobarbital-Na given slowly i.v. A small side branch of the femoral or radial artery is surgically exposed and cannulated for blood pressure recordings using a blood pressure transducer (P23 ID). Heart rate is determined from a conventional ECG lead by a biotachometer. Compounds are administered either intravenously or via a gastric fiberscope, e.g., Olympus XP 10, into the duodenum under visual control. The cardiovascular parameters are registered for a pretest period of 30 min and then during 60 min after intravenous administration or 2 h after intragastric administration of the test drug. Three to six animals are used for evaluation.

Evaluation

Mean values ± SD are calculated for the pretest period and for the cardiovascular effects every min for 5 min after i.v. administration and then every 5 min. After intragastric administration, the values are registered every 5 min up to 30 min and then every 10 min. The values after administration of the test compound are compared statistically with the pretest values using the Student's *t*-test.

Modifications of the Method

Lacour et al. (1993) studied cardiovascular parameters in conscious **cynomolgus monkeys** (*Macaca fascicularis*). A silicone catheter (internal and external diameter 0.64 and 1.19 mm, respectively) was implanted under aseptic conditions into the thoracic aorta via a carotid artery after the monkeys had been anesthetized with 40 mg/kg ketamine and 0.5 mg/kg acepromazine intramuscularly. The vascular catheter (filled with an aqueous solution of 40 % polyvinylpyrrolidone and 20 % heparin) was inserted into a carotid artery. A patch of silicone was sewn around the artery to maintain the catheter in position, the latter being routed subcutaneously and exteriorized at the top of the head into a stainless steel connector. This connector was fixed to the skull with screws and dental cement and sealed with a plug to protect the catheter from damage. The monkeys were permitted a 3-week minimum recovery period. Before the experiment was performed, the monkeys were placed in a primate-restraining chair on several occasions, of gradually increasing duration, for experiment acclimatization.

Pulsatile arterial pressure was recorded by connecting the arterial catheter to a polygraph

via a Statham P23 ID pressure transducer. Mean arterial pressure and heart rate were derived from the pulse pressure signal and recorded. A catheter was inserted acutely into a saphenous vein for administration of compounds.

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Measurement of Cardiac Output and Regional Blood Flow with Microspheres

Purpose and Rationale

The microsphere technique allows the measurement of cardiac output and regional blood flow. Using different radionuclides, repeated determinations are possible. The method is applicable not only for dogs, cats, and minipigs (Hof et al. 1980) but also for rats (McDevitt and Nies 1976; Bonnacrossi et al. 1978; Ishise et al. 1980; Stanek et al. 1985) using microspheres of appropriate size.

Procedure

Male Sprague–Dawley rats weighing 265–375 g are anesthetized with 35 mg/kg i.p. pentobarbital. The right carotid and right femoral arteries are cannulated. Using pressure monitoring, a carotid cannula is manipulated into the left ventricle. Carbonized microspheres (15 ± 5 μ diameter) labeled with ^{85}Sr are drawn into a glass injection chamber and suspended in 0.3 ml 6 % dextran so that each chamber contains 60,000–80,000 microspheres. The radioactivity in each chamber is determined by gamma scintillation counting before and after microsphere injection, the difference being the amount of radioactivity injected. The microspheres are injected into the left ventricle in a total volume of 0.8 ml 6 % dextran over 20 s. Simultaneously, arterial blood from the femoral artery is withdrawn at 0.8 ml/min for 90 s with a syringe withdrawal pump.

Evaluation

This reference blood sample is used to calculate the cardiac output by the formula:

$$\text{Cardiac output} = \text{counts injected} \times \frac{\text{reference sample withdrawal rate}}{\text{reference sample counts}}$$

After obtaining the reference sample, the animals are sacrificed with pentobarbital and the organs dissected, placed in counting vials, and counted for 5 min. Regional distribution of the cardiac output is calculated by comparing the radioactivity in each organ with the total injected radioactivity. Organ flow is determined by multiplying the cardiac output by the fractional distribution of the cardiac output to the organ.

Critical Assessment of the Method

Problems associated with the microsphere technique in rats are the hemodynamic effects of the solutions used to inject the microspheres and the effects of blood withdrawal after repeated determinations (Stanek et al. 1985).

Modifications of the Method

For repeated determinations, other nuclides have been used, such as ^{46}Sc, ^{51}Cr, ^{141}Ce, and ^{125}I (Hof et al. 1980).

Kovách et al. (1992) used up to five radiolabeled microspheres (^{57}Co, ^{113}Sn, ^{85}Sr, ^{95}Nb, and ^{46}Sc) for measurement of regional cerebral blood flow in cats.

Faraci and Heistad (1992) measured blood flow with radioactive microspheres (15 μ diameter) labeled with ^{46}Sc, ^{95}Nb, ^{153}Gd, ^{85}Sr, and ^{141}Ce in anesthetized rabbits.

Grover et al. (1990) and Gross et al. (1992) measured myocardial blood flow in dogs with the radioactive microsphere technique.

Kowallik et al. (1991) measured regional myocardial blood flow with multiple colored microspheres. The method yielded values very similar to those obtained with radioactive microspheres.

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Carotid Artery Loop Technique

Purpose and Rationale

The carotid loop method, originally described by van Leersum (1911) for rabbits, has been used by several authors (e.g., Child and Glenn 1938; Valli et al. 1967; O'Brien et al. 1971; Meyer et al. 1989a, b) for measurement of blood pressure or blood sampling in conscious dogs and sheep (Lagutchik et al. 1992).

Procedure

Male or female inbred Beagle or Labrador–Harrier dogs weighing between 15 and 25 kg are used. They are anesthetized with a bolus injection of 35–40 mg/kg pentobarbital and continued with an infusion of 4–6 mg/kg/h. The animal is placed on a heated operating table. The skin on the ventral side of the neck is carefully shaved and disinfected. The course of the carotid artery is outlined by palpation along the tracheal border. About 2 cm of skin is taken on each side marking the width of the flap. The medial incision is made slightly above the

thyroid cartilage and is extended caudal to a point about 1 cm lateral and 1 cm above the manubrium sterni. The lateral incision again lies about 2 cm from the line of the carotid artery and parallel to it. The lateral incision is only half as long as the medial one. The incisions are made down to the subcutaneous tissue over the platysma muscle. Between the skin and the muscle, the flap is undermined. All bleeding points are carefully clamped and tied.

The subcutaneous tissue, the platysma myoides muscle, and the anterior fascia of the neck are incised in the course of the midline incision down to the plane of cleavage between the sternohyoid and sternomastoid muscles. By blunt dissection, these muscles are separated, disclosing at their depth the neurovascular bundle over which lies the internal jugular vein. The floor of the space so isolated is formed by the longus capitis muscle. By careful dissection, these muscles are separated at least 1 cm above and below the limits of the incision in the skin. The superior thyroid artery marks the uppermost portion of the carotid artery suitable for exteriorization. The plane of cleavage is followed caudal to the origin of the sternocleidomastoid muscle at the manubrium sterni. Throughout the limits of the incision, the artery is dissected free from the internal jugular vein and then from the vagus nerve.

The first step in the exteriorization of the artery is the reapproximation of the muscle borders beneath the vessel by mattress sutures. In order to prevent tension on the completed loop due to contraction of the sternomastoid and sternohyoid muscles, it is important to reapproximate these muscles throughout their course. Sutures are placed at the edges of skin. The tubular flap of skin is then approximated loosely around the carotid artery. It is essential that the skin flap fits loosely around the artery. A continuous suture of fine silk is started at the place where the vessel emerges from the muscle borders. The suture is so placed as to include the artery in a sling of skin which isolates the vessel from the line of suture of the underside of the completed loop. Finally, the proximal and distal quarters of the flap are closed with sutures, while the skin tube is closed with a continuous suture. Antibiotics are given locally and systemically.

One thickness of gauze is placed beneath the loop and along each border a strip of gauze in order to relieve the loop from the pressure caused by the remainder of dressings. Around the neck is wrapped a gauze bandage several turns of which have passed behind the forelimbs in order to prevent the dressing from riding upwards on the animal's neck. Over this is placed a plaster roll protecting the loop from the animal's efforts of scratching. The dressings are changed on the fifth and seventh day when the sutures can be removed.

Blood pressure measurements can be made according to Riva-Rocci's principle by placing a inflatable cuff around the loop.

Critical Assessment of the Method

The carotid artery loop method needs some surgical experience and very meticulous caretaking of the animals.

Modifications of the Method

Lewis et al. (1980) placed a CO_2 sensor using mass spectrometry and its through flow cuvette in a common carotid artery-to-jugular vein loop in anesthetized cats.

Meyer et al. (1989a, b) studied pulmonary gas exchange in panting dogs with an exteriorized carotid artery loop.

Kaczmarczyk et al. (1979) used conscious, chronically instrumented dogs with electric flow probes around the left renal artery and a carotid loop to study postprandial volume regulation.

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Measurement of Heart Dimensions in Anesthetized Dogs

Purpose and Rationale

The measurement of the heart dimensions allows to localize the effect of a drug on the activity of the heart. An ultrasonic technique is used for continuous measurement of left ventricular dimensions. Compounds are tested with potential antianginal activity due to the reduction of left ventricular diameter. The test is used to evaluate the influence of drugs on left ventricular external and internal diameter in anesthetized dogs.

Procedure

Male or female Beagle or Labrador–Harrier dogs weighing between 15 and 25 kg are used for the test. The dog is anesthetized by intravenous injection of 35–40 mg/kg sodium pentobarbital followed by subcutaneous injection of 2 mg/kg morphine. Respiration is maintained through a tracheal tube with N_2O/O_2 (3:1) using a positive pressure respirator.

Implantation of Ultrasonic Transducers

Ultrasonic transducers are constructed and implanted as described by Stinson et al. (1974).

To measure left ventricular external diameter (LVED), two ultrasonic transducers are fixed to the left ventricular wall. One crystal is sutured to the posterior wall within the rectangular area formed by the left circumflex coronary artery and the left posterior descending artery. The other one is placed near the first diagonal branch of the left anterior descending coronary artery. Exact positioning is assured with an oscilloscope.

To measure left ventricular internal diameter (LVID), the transducers are placed in the same anatomical area as for the epicardial crystals. However, they are pushed through the wall of the left ventricle through stab wound incisions. The crystals are positioned across the greatest transverse diameter of the left ventricle, one on the anterior and the other on the posterior endocardial wall.

Bleeding during the implantation procedure is controlled by umbilical tapes around the cranial and caudal veins and by purse-string sutures at the implantation sites. The pericardial incision and the chest are closed by sutures, and the transducer wires are connected to the recording equipment.

In each dog, either LVED or LVID is measured together with the other hemodynamic parameters.

Preparation for Hemodynamic Measurements

Blood pressure is recorded through a cannulated femoral artery by a pressure transducer (Statham P23 DB).

For determination of LVP, a Millar microtip catheter (type PC 350) is inserted via the left A. carotis communis. LVEDP is measured on a high-sensitivity scale. From the pressure curve, d*p*/d*t*max is differentiated and heart rate is calculated.

Hemodynamic parameters are recorded continuously during the whole experiment.

Experimental Course

When stable hemodynamic conditions are achieved for at least 30 min (control values), the test substance is administered by intravenous or intraduodenal injection.

Readings are taken at times 0, 15, 30, 45, 60, 75, 90, and 120 after drug administration. Left ventricular dimensions are measured at the end of the diastole and systole.

Characteristics:

- Blood pressure
  - Systolic blood pressure
  - Diastolic blood pressure
- Left ventricular pressure, LVP
- Left ventricular end-diastolic pressure
- Left ventricular contractility, d*p*/d*t*
- Heart rate, HR
- Left ventricular external diameter, LVED
- Left ventricular internal diameter, LVID

Evaluation

Hemodynamic parameters, LVED, and LVID [mm] are determined.

Changes in parameters after drug administration are compared to control values obtained during the 30-min predrug period.

Statistical significance is assessed by means of the paired *t*-test.

Since a change in the diameter of the left ventricle is a reasonable accurate index of left ventricular volume, a reduction of LVED or LVID with no change in d*p*/d*t* and HR can be considered as a strong indicator for "venous pooling" and thus an antianginal activity of a compound.

Scores are allotted relative to the efficacy of standard compounds assessing the intensity as well as the duration of the effect.

Standard data:

		LVED [mm]		LVID [mm]	
Nitroglycerin	0.005 mg/kg, i.v.	−0.9	20 min	−1.2	30 min
Isosorbide dinitrate	0.1 mg/kg, i.v.			−0.6	120 min
Molsidomine	0.2 mg/kg, i.v.	−2.1	>60 min	−1.4	>120 min
Nifedipine	0.1 mg/kg, i.v.			+1.2	120 min

Modifications of the Method

Novosel et al. (1992) measured the dimensions of the right ventricle with microsonometry in anesthetized rabbits.

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Telemetric Monitoring of Cardiovascular Parameters in Rats

Purpose and Rationale

Radiotelemetry allows the recording of cardiovascular parameters in conscious, free-moving animals. Several authors (Brockway et al. 1991; Mattes and Lemmer 1991; Guiol et al. 1992; Morimoto et al. 1992; Basil et al. 1993; Brockway and Hassler 1993; Lemmer et al. 1993, 1994, 1995; Calhoun et al. 1994; Diamant et al. 1993; Kramer et al. 1993a, 1995; Griffin et al. 1994; Kuwahara et al. 1994; Sato et al. 1994, 1995; van den Buuse 1994; Kinter 1996; Becker et al. 1997; Witte et al. 1998) used commercially available systems with some modifications to study the circadian rhythm of blood pressure and the influence of drugs on heart rate, blood pressure, and motility in rats.

Procedure

The telemetry and data-acquisition system (e.g., Data Sciences International, Inc., St Paul, MN) consists of four parts:

1. The implantable transmitter, which measures the pressure. This device contains a highly stable ion implant, semiconductor, strain-gauge sensor, and battery-powered electronics to process the information from the pressure sensor and to telemeter it from within the animal. Arterial pressure is transmitted to the sensor via a 0.7-mm-diameter, fluid-filled catheter.
2. The receiver which detects the signal from the implanted transmitter and converts it to a form readable by computer.
3. The pressure reference module, which measures atmospheric pressure to allow for the telemetered absolute pressure to be converted to a gauge pressure.
4. The data-acquisition software, which accepts data from the reference module and the receivers, filters corrupt samples from the incoming data stream, converts the telemetered pressure to millimeters of mercury, subtracts atmospheric pressure from the telemetered pressure, and stores the data for retrieval, plotting, and analysis.

Under pentobarbital anesthesia, the telemetry transmitter is implanted into rats. The descending aorta is exposed between the renal arteries. A vascular clamp is made by putting two surgical threads on the proximal and distal part of the artery. The catheter tip is inserted through an incision in the vessel. A drop of cyanoacrylate glue is applied to the dried entry point. The transmitter is sutured to the abdominal musculature.

Evaluation

Data from individual animals are recorded over long periods of time which allow the investigator to follow the circadian rhythm under several experimental conditions.

Modifications of the Method

Hess et al. (1996) monitored pulmonary arterial pressure in freely moving **rats** by inserting the sensing catheter of a telemetric system through a small hole and pushing it into the pulmonary artery.

Further cardiovascular studies in rats using the telemetric system were reported by Sgoifo et al. (1998) and Webb et al. (1998).

Kramer et al. (1993b) used telemetry to record electrocardiogram and heart rate in freely moving **mice**.

Carlson and Wyss (2000) used small telemetry probes for long-term recording of arterial pressure and heart rate in mice after implantation to the carotid artery or the abdominal aorta.

DePasquale et al. (1994) used radiotelemetry to monitor cardiovascular function in conscious **guinea pigs**.

Telemetric ECG recordings in **cardiomyopathic hamsters** were reported by Desjardins et al. (1996).

Van den Buuse and Malpas (1997) studied cardiovascular parameters in **rabbits** by radiotelemetry.

Astley et al. (1991) and Smith et al. (1993) used telemetric systems to monitor cardiovascular responses in **baboons**.

Schnell and Wood (1993) measured blood pressure and heart rate by telemetry in conscious, unrestrained **marmosets**.

An ultrasonic blood flowmeter telemetry system for **cats** and rabbits has been described by Yonezawa et al. (1989, 1992).

Telemetry was used by Symons et al. (1992) to monitor the severity of events representing myocardial dysfunction in **miniswine**.

Savory and Kostal (1997) applied the telemetric system for chronic measurement of cardiovascular and other parameters in **chicken**.

Radiotelemetry has also been used for other pharmacological experiments, such as field potential analysis by radioelectroencephalography (see section "EEG Analysis from Rat Brain by Telemetry" in chapter "► Effects on Behavior and Muscle Coordination"), step-through passive avoidance (see section "Step Through" in chapter "► Behavioral Methods Used in the Study of Learning and Memory"), shock-prod burying test in rats (see section "Progressive Ratio Procedure" in chapter "► Tests for Anxiolytic Activity"), measurement of body temperature (see section "Antipyretic Testing in Rats" in chapter "► Anti-Pyretic Activity"), and motility in rats and mice (Clement et al. 1989; Guillet et al. 1990; Diamant et al. 1993; van den Buuse 1994).

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Cardiovascular Effects After Intracerebroventricular Administration

Purpose and Rationale

Several drugs, like α_2-adrenergic agonists, act primarily at central sites. Their effects can be most clearly demonstrated after injection into the cerebroventricular system. The first experiments have been performed in cats. The method has been adapted to rats.

Procedure

Rats of either sex weighing 250–350 g are anesthetized with 100 mg/kg hexobarbital i.p. The scalp is cut in a sagittal line. With a dental drill, a hole of 1–1.5 mm diameter is drilled through the cranial bone 1 mm lateral and 2 mm caudal of the bregma. A PVC is introduced perpendicular to the bone to a depth of 3 mm in order to reach the lateral cerebral ventricle. The catheter is fixed with dental cement and the wound closed. Test substances are administered through the catheter. To measure blood pressure, one catheter is placed in one carotid artery and connected to a Statham transducer. Blood pressure and heart rate are recorded on a polygraph over a period of at least 30 min. For long-acting drugs, registration periods up to 2 h are necessary. After the experiment, the animal is sacrificed and the brain removed to confirm the site of injection.

Evaluation

Systolic and diastolic blood pressure as well as heart rate after intracerebroventricular injection are expressed as percentage of pretreatment values. The response is compared with the standard clonidine which is effective in doses of 4–60 μg.

Modifications of the Method

Based on the work of Feldberg and Sherwood (1954) and Hayden et al. (1966), Mastrianni et al. (1986) developed an intracerebroventricular perfusion system for the study of centrally acting antihypertensive drugs in the rat. The antihypertensive effect of clonidine could be observed over several hours.

Methods used to detect central hypotensive activity of drugs have been reviewed by Timmermans (1984).

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Influence on Orthostatic Hypotension

Purpose and Rationale

Orthostatic hypotension with dizziness up to unconsciousness is a syndrome occurring in many human individuals. Moreover, several drugs are known to cause orthostatic hypotension. In several animal species, such as rabbit, cat, and dog, this syndrome can be evoked by changing the usual horizontal position into a vertical position with the head upwards using a tilting table.

Procedure

Cats of either sex weighing 2.0–3.0 kg are temporarily anesthetized with ether. Anesthesia is maintained by intravenous injection of 70 mg/kg chloralose. The animal is fixed with its legs on a heated operating table which can be tilted by 90°. The carotid artery is cannulated for measuring blood pressure through a Statham P23 DB transducer on a 6-channel Hellige recorder. The femoral vein is cannulated for injection of the test compound. After the blood pressure is stabilized for 30 min, the animal is quickly tilted to a vertical position for 1 min. Due to the change of position and gravitational force, there is a rapid fall in blood pressure which recovers as soon as the animal is restored to its original position. After taking the control reading, the test compound is administered intravenously and the same procedure is repeated. The fall in blood pressure is recorded.

Evaluation

A significant increase in postural hypotension with respect to the control would indicate that the test compound may produce orthostatic hypotension in human. Moreover, some compounds, like sympathomimetics, can reduce or prevent postural hypotension.

Modifications of the Method

Sponer et al. (1981) described a method for evaluating postural hypotension in conscious **rabbits** placed on a tilting table whereby blood pressure was measured from the central artery of the ear.

Takata et al. (1999) reported a rabbit model for evaluation of chlorpromazine-induced orthostatic hypotension.

Humphrey and McCall (1982) described a model for predicting orthostatic hypotension during acute and chronic antihypertensive drug therapy in **rats** anesthetized with chloralose, urethane, and pentobarbital using a heated tilting table.

Lee et al. (1982) evaluated postural hypotension induced by drugs in conscious restrained normotensive rats. Blood pressure was recorded after cannulation of the femoral artery under light ether anesthesia. A special tilting table was build for simultaneous studies in four rats.

Martel et al. (1996, 1998) studied the phenomenon of cardiovascular deconditioning observed in crew members of space flights in rats after tail suspension.

Socci et al. (2000) studied cardiovascular responses to simulated microgravity in Sprague–Dawley rats. Microgravity is known to induce orthostatic intolerance and baroreflex impairment in astronauts. The authors used 30° head-down tilt, 24-h whole-body suspension, or 7-day tail suspension to mimic microgravity and to find treatment ameliorating the symptoms.

Baum et al. (1981) studied antihypertensive and orthostatic responses to drugs in conscious **dogs**. A catheter was placed in the subclavian artery for measurement of blood pressure and exteriorized at the back of the neck some days prior to the experiment. The animals were placed into a sling and tilted to the 90° upright position for periods of 60 s every hour by lifting their forelimbs. Blood pressure response before and after treatment with test drugs was measured.

A nonhuman **primate** model for evaluating the potential of antihypertensive drugs to cause orthostatic hypotension was described by Pals and Orley (1983) Polyvinyl catheters were implanted in the abdominal aorta and the vena cava via an external iliac artery and vein to **cynomolgus monkeys** during ketamine anesthesia. The catheters were routed subcutaneously from the groin area to the top of the head and exteriorized. After recovery the animals were placed in restraining chairs allowing the change from vertical to horizontal position.

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Bezold–Jarisch Reflex

Purpose and Rationale

The circulatory collapse after intravenous injection of veratrine has been first described in cats and is known as Bezold–Jarisch reflex (von Bezold and Hirt 1867; Jarisch and Richter 1939a, b; Jarisch 1940; Aviado and Guavera-Aviado 2001).

Fleckenstein et al. (1950) recommended this as a suitable animal model of shock.

The original observation was a triphasic blood pressure response in cats or dogs characterized by a short-lasting fall in blood pressure accompanied by bradycardia, followed by a short-lasting increase and then a long-lasting decrease of blood pressure after intravenous injection of veratridine or other veratrum alkaloids.

Kalkman et al. (1984) showed that three distinct subtypes of serotonergic receptors mediate the triphasic blood pressure response to serotonin observed in the Bezold–Jarisch reflex.

The Bezold–Jarisch reflex has been studied in several species, such as **cats** (Takei et al. 1995; Vayssettes-Couchay et al. 1997), **dogs** (Zucker and Cornish 1981; Barron and Bishop 1982; Harron and Kobinger 1984; Giles and Sander 1986; Baugh et al. 1989; Watson et al. 1995), **ferrets** (Andrews and Bhandari 1993), **rabbits** (Chen 1979), guinea pigs, **rats** (Fozard 1984; Gylys et al. 1988; Cohen et al. 1989; Blower 1990; Miyata et al. 1991; Turconi et al. 1991; Matsumoto et al. 1992; Meller et al. 1992; Robertson et al. 1992; Kishibayashi et al. 1993; Geissler et al. 1993; Haga et al. 1994; Hegde et al. 1995; Eglen et al. 1995; Göthert et al. 1995; Delagrange et al. 1996; De Vries et al. 1997; Malinowska et al. 2001; Godlewski et al. 2003), and **mice** (Eglen et al. 1994; Middlefell et al. 1996), whereby species differences have been observed (Yamono et al. 1995).

In cats and dogs, the Bezold–Jarisch reflex was elicited by veratrine and veratridine but also by capsaicin and the 5-HT_3 receptor agonists 2-methyl-5-HT, phenylbiguanide, chlorophenylbiguanide, and serotonin itself.

In rats, mostly 5-HT or 2-methyl-5-HT were used as stimuli to characterize 5-HT_3 receptor antagonists.

Procedure

Male Sprague–Dawley rats weighing 250–380 g are given food and water ad libitum, except those used for intraduodenal drug administration; these rats are deprived of food overnight. The animals are anesthetized by intraperitoneal injection of 1.5 g/kg urethane. Body temperature is maintained at 37 °C by placing the animal on a heating pad. The left jugular vein or duodenum, trachea, and left femoral vein are cannulated for drug administration (i.v. or i.d.), facilitation of respiration, and injection of 2-methyl-5-HT, respectively. Heart rate is derived from a limb lead II ECG monitored via subdermal platinum electrodes and is recorded with amplifiers on a polygraph. A dose–response curve to 2-methyl-5-HT (5–100 μg/kg, i.v.) is constructed in each rat to establish a submaximal dose (usually 10 or 20 μg/kg, i.v.) which elicits a reproducible bradycardic response. Each rat receives then a single dose of test drug or standard and is then challenged with 2-methyl-5-HT at 5, 15, 30, 60, 120, 180, 240, 300, 360, 420, and 480 min post dosing. A separate group of rats receiving vehicle (saline for i.v., deionized water for i.d.) is similarly tested in each study.

Evaluation

Duration of action of the compounds is assessed by determining the period of time for which the inhibitory effects remain significantly different from vehicle controls. Statistical analysis of the data is performed by a repeated measure analysis of variance (ANOVA) followed by pairwise comparisons against control at each time period using Fisher's multiple comparison test.

Modifications of the Method

Harron and Kobinger (1984) used capsaicin to elicit the Bezold–Jarisch reflex in anesthetized artificially respirated dogs pretreated with a beta-adrenoceptor antagonist to evaluate the activity of clonidine-like drugs on central alpha$_2$-adrenoceptors after intracisternal administration.

The Bezold–Jarisch reflex in rats has been used for evaluation of 5-HT_3 receptor agonists (Rault et al. 1996; López-Tudanca et al. 2003).

Rocha et al. (2003) found an enhancement of the Bezold–Jarisch reflex in the acute phase of myocardial infarction of the anesthetized rabbit.

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Endotoxin-Induced Shock

Purpose and Rationale

Many bacterial infections as well as allergic reactions are known to induce pathophysiological events that may lead to shock in man. When experimental animals are injected with endotoxin and galactosamine, shock and death occur in all untreated animals 5–7 h after injection. The endotoxin-induced shock is marked by pulmonary embolism, bronchospasm, and renal failure. Bacterial lipopolysaccharides (endotoxins) play an important role in the pathogenicity of Gram-negative infections.

The reactivity of animals to endotoxin may be enhanced by simultaneous administration of galactosamine. Galactosamine is a specific hepatotoxic agent that leads to early metabolic alterations and consequent cellular liver damage. The following procedure is used to detect compounds that prevent the occurrence of endotoxin-induced shocks.

Cardiovascular parameters of endotoxin-induced shock are greatly influenced by various anesthetics. For this reason, a model was proposed by Brackett et al. (1985) and Schäfer et al. (1987) to study the circulatory shock pattern after endotoxemia in conscious unrestrained rats.

Procedure

Male Sprague–Dawley rats weighing 300 ± 10 g are anesthetized with 5 % enflurane. A tracheal cannula is connected to a rodent respirator delivering 2 % enflurane. Via the right jugular vein, the tip of one catheter is placed just adjacent to the right atrium for injection of endotoxin, monitoring of central venous pressure, and rapid injection of room-temperature saline to produce thermodilution curves for calculation of cardiac output. The right carotid artery is cannulated with a thermistor–catheter combination for measurement of thermodilution cardiac output curves and aortic blood pressure. The thermistor tip is placed in the aortic arch just distal to the aortic valve. The catheters are guided under the skin exiting through the back of the neck just below the base of the skull.

The animals are allowed to regain consciousness and are then placed in cages that allow unrestrained movements about the cage at all times throughout the study with no further handling. The experimental animals receive a 20-s infusion of 40 mg/kg endotoxin (*E. coli*, Difco) being paired with sham animals with identical catheters but receiving an equal volume of saline. Test compounds are injected intravenously 10 min prior to endotoxin injection. Cardiac outputs are measured using the thermodilution technique by rapidly injecting a volume calculated to deliver 100 µl of room temperature saline to the circulatory system. Central venous and aortic blood pressure and heart rate are continuously monitored for the following 4 h. Cardiac output measurements are made 5, 15, 30, 60, 120, 180, and 240 min after endotoxin. At the end of the study, the animals are sacrificed and the catheters checked visually to ensure proper placement.

Evaluation

Central venous pressure, arterial pressure, and cardiac output of drug-treated animals receiving endotoxin are compared with animals receiving endotoxin only and saline–sham-treated animals. Furthermore, cardiac index, total peripheral resistance, and stroke volume are calculated. The small intestines of all rats are examined for severity of hemorrhage using a five-point scale. Repeated-measures analysis of variance is used to analyze the data.

Modifications of the Method

Lindenbaum et al. (1990) studied the effect of *E. coli* endotoxin on cardiovascular parameters of anesthetized **dogs**. Inhibition of the deterioration of metabolic functions and improvement of cardiovascular parameters were found after cocarboxylase treatment.

Endotoxin-induced shock has been tested in **mice** (Galanos et al. 1979). Groups of 10 male C57BL/6 mice weighing 20–22 g are injected intravenously with a mixture of 0.01 μg of *Salmonella* abortus equi lipopolysaccharide and 7.5–15 mg galactosamine in 0.02 ml phosphate-buffered saline. The test compound is administered either intravenously at the same time or orally 45 min prior challenge. Twenty-four hours later, the number of surviving mice is determined.

Metz and Sheagren (1990) reviewed the effects of ibuprofen in animal models of septic shock.

Von Asmuth et al. (1990), Luongo et al. (1998), and Cuzzocrea et al. (2004) described a zymosan-induced shock model in **mice**. Male CD mice (20–22 g) were treated intraperitoneally with zymosan (500 mg/kg, suspended in saline solution) or with zymosan and drug (rosiglitazone 3 mg/kg, intraperitoneally) at 1 and 6 h after zymosan. Eighteen hours after administration of zymosan, animals were assessed for nonseptic shock. Clinical severity of systemic toxicity was scored for the whole experimental period (12 days) in the mice after zymosan or saline injection on a subjective scale ranging from 0 to 3: 0 = absence, 1 = mild, 2 = moderate, and 3 = serious. The ranging scale was used for each of the toxic signs (conjunctivitis, ruffled fur, diarrhea, and lethargy) observed in the animals. The final score was the sum of the single evaluation (maximum value 12).

Overbergh et al. (2003) studied acute shock induced by antigen in NOD mice. The 8-week-old NOD, BALB/c, and C57BL/6 mice were immunized by injection of 100 μg antigen [hen egg-white lysozyme (HEL), GAD65 (p524–542) (SRLSKVAPVIKARMMEYGTT), bovine insulin B (ins-B) chain, heat shock protein (hsp)-65, PLP peptide (amino acids 135–151), ovalbumin, keyhole limpet hemocyanin (KLH), and tetanus toxin] emulsified at a 1:1 concentration in complete Freund's adjuvant (CFA) or incomplete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA) in the hind footpads. NOD–*SCID* mice were injected with 100 μg HEL antigen suspended in CFA. All mice were reinjected with the same antigen 3 weeks later in a similar manner. Clinical evolution and survival rate after sensitization with various peptides were monitored in different mouse strains. Shock was characterized by piloerection; prostration; erythema of the tail, ears, and footpads; and dyspnea with shallow breathing. Serum for antibody measurement and spleens for quantification of mRNA levels were collected before immunization and again before booster administration.

Baldwin et al. (1991) tested the effect of polymyxin B on experimental shock from meningococcal lipooligosaccharide and *Escherichia coli* lipopolysaccharide endotoxins in anesthetized **rabbits**.

Muacevic and Heuer (1992) tested the effect of platelet-activating factor antagonists in anesthetized rats.

Otterbein et al. (1993) tested the effects of peptides on survival of mice injected with 50 mg/kg lipopolysaccharide endotoxin in mice and on survival of rats with fecal peritonitis.

Mountz et al. (1995) reported an increased susceptibility of fas mutant **MRL-Ipr/Ipr mice** to staphylococcal enterotoxin B-induced septic shock.

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Hemorrhagic Shock

Purpose and Rationale

Hemorrhagic shock is one of the most severe consequences of accidents. Several animal models in various species have been developed to resemble the conditions in man and to test therapeutic or prophylactic measures (Lamson and de Turk 1945; Selkurt and Rothe 1961; Mills 1967). A method for hemorrhagic shock in anesthetized as well as in unanesthetized rats has been described by van der Meer et al. (1987). Experimental hemorrhagic shock is defined as a situation in which the cardiovascular system, after a period of hypovolemia followed by complete reinfusion of the shed blood, gradually deteriorates ending in the death of the animal.

Procedure

Female rats weighing 170–190 g are anesthetized by i.p. injection of sodium pentobarbital, 25 m/kg, followed after 20 min by 20 mg/kg, and kept in a chamber at 30 °C and relative humidity

over 80 %. The left femoral vein is cannulated for application of the test drug. The right common iliac artery is cannulated and the cannula (polyvinyl chloride, 14 cm long, inner diameter 2 mm) is filled with heparin and exteriorized in the neck. After intra-arterial injection of 0.2 ml heparin 500 IU/ml, the cannula is connected to a siliconized calibrated glass reservoir (inner diameter 18 mm), the height of which can be changed to adjust the surface of the shed blood to a fixed level.

The test drug is injected i.v. 5 min prior to bleeding. Bleeding is performed against (at heart level) 30 mmHg for 1 h, 25 mmHg for 0.5 h, 30 mmHg for 1 h, 25 mmHg for 0.5 h, and finally 30 mmHg for 1 h. The shed blood is partially taken up again spontaneously. After 4 h, reinfusion is started by increasing the pressure to 60 mmHg for 5 min, to 80 mmHg for 5 min, and (if necessary) to 100 mmHg. During the hypovolemic phase, respiration becomes gradually slower. If respiration arrest is imminent, 0.5 ml 5 % glucose is injected intra-arterially, thus avoiding death during the period of hypovolemia. Practically all rats die at an average of 4 h after complete reinfusion.

Evaluation

Survival time is taken as the time between complete reinfusion and death. Average survival time of treated animals is compared with that of controls. Furthermore, after autopsy the number of gastrointestinal lesions, subendocardial hemorrhage, kidney tubular necrosis, and liver cell necrosis are registered by histological examination.

Critical Assessment of the Method

In spite of the fact that hemorrhagic shock does not reflect the situation of traumatic shock in man in every aspect, the condition is close enough to use the model for testing compounds which potentially inhibit or ameliorate shock in man.

Modifications of the Method

A method to study hemorrhagic shock in dogs has been described in detail by Mills (1967). Large dogs weighing 20–30 kg are anesthetized by an i.v. injection of 25 mg/kg sodium pentobarbital. The animals are respirated by means of a Harvard respirator set at a stroke volume of 400 ml and a rate of 20 respiration/min. Blood pH is regulated between 7.37 and 7.42 by varying the gas flow between 100 % O_2 and a mixture of 95 % O_2 and 5 % CO_2. Central arterial blood pressure is recorded by inserting a catheter through one femoral artery to the aortic arch. Pulmonary artery pressure is measured by inserting a PE-50 catheter through a small neck vein, reaching the right ventricle and allowing to float into the pulmonary artery. The right atrial catheter is also inserted through a small neck vein. After the chest is opened, the left atrial catheter is tied in place through a small opening in the left atrial appendage. Blood flow is measured in the ascending aorta (cardiac output), carotid, superior mesenteric, renal, and femoral arteries using electromagnetic flowmeters. Furthermore, pulse rate is monitored from the electrocardiogram.

The test drug is injected i.v. 10 min prior to bleeding. Blood is removed either at a specific volume or until a selected reduction of blood pressure has occurred. The cardiovascular parameters of treated animals are compared with those of controls.

Shock associated with hemoconcentration was produced in dogs (Davis 1941) by bleeding from the carotid artery and injections of 25 % sodium chloride solution subcutaneously in doses of 25 ml.

The effect of insulin on glucose uptake in the soleus muscle of rats during hemorrhagic shock was studied by Chaudry et al. (1975).

Bauer et al. (1995) used hemorrhagic shock in rats to evaluate the influence of interleukin-1 on leukocyte–endothelial cell interactions and the microcirculation in the liver by means of intravital microscopy after application of an interleukin-1 receptor antagonist.

Kitajima et al. (1995) studied gastric mucosal injury induced by hemorrhagic shock in rats.

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Tourniquet Shock

Purpose and Rationale

Compression of extremities in man by heavy objects for periods of several hours results in the so-called crush syndrome. The rescued individual shows immediately a favorable response to therapy, but within a few hours, symptoms of shock develop followed by signs of progressive renal damage leading to death (Duncan and Blalock 1942). Moreover, arterial bleeding after accidents needs the applications of tourniquets. During surgical procedures on extremities, a tourniquet may be necessary (Wilgis 1971), the time of which has to be limited in order to avoid fatal consequences. The pathophysiological mechanisms of tourniquet-induced shock remain still to be elucidated. Nevertheless, animal models in rats (Chandra and Dave 1970), rabbits (Little 1974), and dogs (Goto et al. 1988) had to be developed to evaluate drugs capable to inhibit the fatal consequences of crush and tourniquet shock.

Procedure

Wistar rats of either sex weighing 250–280 g are anesthetized with phenobarbital. The tourniquets consist of rubber tubes (internal diameter 4 mm, external diameter 5.8 mm). Both tights are fastened by the rubber tubes, and the pressure which is monitored by a miniature pressure sensor and an amplifier (e.g., Kyowa Electronic Instruments Co, Tokyo) is adjusted to 1.5 kg/cm^2. The rubber tubes are knotted and the sensor removed. After 3 h the animals are treated with the test compound or the control solution. The tourniquet is left in place for 6 h while the animals remain under pentobarbital anesthesia. Then, the rubber tubes are removed, and the rats are returned to their cages. Within a few min, the reperfused hind limbs, which have been pale blue, turn pink. The animals are then allowed free access to food and water. Blood is withdrawn at different intervals during the tourniquet and afterwards for measurement of hematocrit, transaminases, urea nitrogen, and total protein. Time to death is registered.

Evaluation

Statistical evaluation of the survival intervals is performed with the log-rank test according to

Peto et al. (1976). Blood chemical data are analyzed using the Kruskal and Wallis (1952) rank-sum test. Multiple comparisons are corrected by the Bonferroni's method (Dunn 1961).

Critical Evaluation of the Method

These methods are valuable to find drugs effective in this life-threatening situation.

Modifications of the Method

Ghussen et al. (1979) studied the effect of methylprednisolone on the experimental tourniquet shock in **dogs**.

Haugan and Kirkebo (1984) used a model in anesthetized **rats** with tourniquet shock by bilateral hind-limb occlusion for 3 1/2 h and burn shock by scalding the hind 50 % of the body surface for 30 s in 90 °C water.

Horl and Horl (1985) investigated the effect of tourniquet ischemia on carbohydrate metabolism in **dog** skeletal muscle

Sáez et al. (1982) followed the time course of appearance of lactic dehydrogenase enzymes in the serum of **rats** after different periods of ischemia by bilateral application of rubber band tourniquets to the hind legs.

Sáez et al. (1986) studied the effects of allopurinol on biochemical changes of the gastrocnemius muscle in rats subjected to tourniquet shock followed by reperfusion.

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Heatstroke

Purpose and Rationale

Heatstroke is a medical emergency where quick diagnosis and treatment of victims are essential for positive prognosis. Several animal models have been established by investigators in heat-related studies. Rats (Francesconi and Mager 1978; Hubbard et al. 1977, 1979; Kielblock et al. 1982), rabbits (Shih et al. 1984), dogs (Bynum et al. 1977), and sheep (Tayeb and Marzouki 1990) are considered to be the most suitable models because of their similarity to man in response to high temperature.

Procedure

Male Sprague–Dawley rats weighing 450–550 g are fasted 18–24 h before the experiment. For prevention studies, the animals are treated subcutaneously 1 h before either being restrained in an appropriate wire cage which is placed into an environmental chamber set at 41.5 °C ambient temperature or being exercised in a motor-driven treadmill. Core temperature (rectal probe inserted 6.5 cm) is measured using copper/constantan thermocouples in conjunction with a thermocouple reference oven and a 10-channel data-acquisition system with a teletype printout. After reaching exhaustion or a predetermined core temperature, all rats are monitored at 26 °C ambient temperature while resting in plastic cages lined with wood shavings. After recovery, animals are returned to their cages and allowed water but no food for 24 h.

Evaluation

LD_{50} values are determined in treated and control animals.

Modifications of the Method

Kielblock et al. (1982) analyzed cardiovascular function by direct recording of arterial blood pressure and ECG analysis.

Francesconi and Mager (1978) studied pathochemical indices, such as serum lactate concentration, potassium levels, and plasma creatine phosphokinase.

Kregel et al. (1988) investigated peripheral vascular responses to hyperthermia in the rat by implantation of Doppler flow probes on the superior mesenteric, left iliac or left renal, and external caudal arteries. They concluded that a selective loss of compensatory vasoconstriction triggers the cascade of events that characterize heatstroke.

Shido and Nagasaka (1990) studied thermoregulatory responses to acute body heating in rats acclimated to continuous heat exposure. Indirect external warming was performed by raising the jacket water temperature surrounding the calorimeter from 24 °C to 39 °C. Intraperitoneal heating was made through an electric heater implanted chronically in the peritoneal cavity.

Chiu et al. (1995) reported an increased survival in rat heatstroke by reducing hypothalamic serotonin release after administration of interleukin-1 receptor antagonist.

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α- and β-Adrenoreceptors in the Mouse Iris

Purpose and Rationale

A simple method to test mydriatic substances is the test on the mouse pupil as described by Pulewka (1932). The diameter of the pupil is narrowed by intensive light illumination. A dose-dependent increase of pupil diameter can be achieved by intraperitoneal application of atropine and synthetic mydriatics (Ing et al. 1945; Burn et al. 1950). The mydriatic effect of hexamethonium analogues has been measured by Blackman et al. (1956). Mydriasis is induced by norepinephrine, epinephrine, and isoproterenol and can be antagonized by α- or β-blockers (Freundt 1965).

Procedure

Male mice weighing 15–20 g are used. They are kept for at least 30 min in separate beakers under bright illumination before the pupil diameters are measured with a dissecting microscope containing an arbitrary scale in the eyepiece. To make the illumination as uniform as possible, the beakers containing the mice are placed beneath long low-power fluorescent tubes and on top of glossy white paper. The pupil diameter is measured in mm before and at various time intervals after treatment. Groups of 5–10 mice are used for each dose of compound and for vehicle control.

To test sympatholytic activity, various doses of the α- or β-blocker are injected subcutaneously 30 min prior to intravenous injection of 0.1 mg/kg norepinephrine, or 0.05 mg/kg epinephrine, or 20 mg/kg isoproterenol. The effect of norepinephrine is blocked by α-blockers, but not by β-blockers; the effect of epinephrine by both α- and β-blockers; and the effect of isoproterenol by β-blockers, but not by α-blockers.

Evaluation

The mean values of diameters in the groups treated with α- or β-blockers are compared with those of animals treated with norepinephrine, epinephrine, or isoproterenol only.

Modifications of the Method

Edge (1953) used mydriasis in the mouse as a quantitative method of estimating parasympathetic ganglion block.

Håkanson et al. (1987) used the isolated iris sphincter of pigmented rabbits to test multiple

tachykinin pools in sensory nerve fibers. The eyes were taken out within 1 min after sacrifice and opened by an incision 2–3 mm posterior to the limbus, followed by excision of the iris from the ciliary margin. The iris sphincter muscle was then opened, cut in half, and mounted vertically on a Perspex holder in a 7-ml tissue bath maintained at 35 °C. The mechanical activity after electrical stimulation was recorded isometrically using a force displacement transducer and a polygraph.

Kern (1970) used isolated sphincter and dilator muscles from human eyes obtained at autopsy for studies on sympathomimetics and adrenergic blocking agents. Cholinotropic and α- and β-adrenergic receptors were identified.

Responses to bradykinin and/or capsaicin of the isolated iris sphincter were considered to be mediated by substance P released from the trigeminal nerve (Ueda et al. 1984).

Pupillary dilatation can be used as an index for central nervous system α_2-adrenoreceptor activation (Koss 1986).

Clonidine induces mydriasis which is mediated by α_2-adrenoreceptors located in the brain (Berridge et al. 1983; Hey et al. 1985). Blockade of presynaptically located α_2-adrenoreceptors is considered as a possible mechanism for antidepressant drugs. Mianserin was able to antagonize clonidine-induced mydriasis in the rat.

Gower et al. (1988) studied a large number of psychotropic drugs in this model with the aim to reveal in vivo α_2-adrenoreceptor blocking effects of new compounds.

Male Wistar rats weighing 230–300 g were anesthetized with pentobarbital, 60 mg/kg i.p., and a polyethylene catheter was inserted into the femoral vein for drug administration. The rat's head rested on the base platform of a binocular Olympus microscope positioned so that the pupil diameter of the right eye could be measured by means of a micrometer inserted into one eyepiece of the microscope. A constant light intensity was maintained throughout the experiment. Rats were first injected with saline 25 min after anesthesia induction. The pupil diameter was measured 1 min after injection. Five min after measurement, mydriasis was induced by clonidine (0.1 mg/kg, i.v.) and the diameter was measured 1 min after injection. This was followed by the test compound, injected at 6-min intervals at increasing doses. The pupil was measured at 1 min after each injection. The dose inhibiting 50 % of the clonidine-induced mydriasis (ID_{50}) was determined per rat from the cumulative dose–response curve.

Savontaus et al. (1997) studied the effect of an imidazoline derivative against detomidine-induced mydriasis in anesthetized rats.

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α_2-Adrenoreceptor Blockade Measured In Vivo by Clonidine-Induced Sleep in Chicks

Purpose and Rationale

In young chicks, clonidine causes a loss of righting reflex which is antagonized by mianserin (Pommier et al. 1982). This phenomenon was used to measure α_2-adrenoceptor blockade in vivo by Gower et al. (1988).

Procedure

Male white Leghorn chicks are used either a few hours after hatching or 1 or 2 days later. Clonidine-induced loss of righting reflex (sleep) is determined with eight animals at a time. Two animals are treated with placebo and 2 with each of 3 dose levels of the test compound. Tests with groups of 8 animals are continued until 10 animals are tested per dose level or placebo treatment. The chicks are marked with ink and injected intraperitoneally with placebo or the test compound. Ten min later, 1.2 mg/kg clonidine is injected into a leg muscle, and the animals are placed individually in small Makrolon cages. The beginning of sleep time is defined as the moment at which the animals can be placed on their back and remain in this position. Sleep time is recorded until they return to their feet spontaneously or another attempt to put them on their back fails. Sleep time is recorded for a maximum period of 30 min.

Evaluation

Statistical evaluations of differences in median sleeping times are done with the Mann–Whitney *U*-test. Dose–response relations for various drugs can be calculated.

Critical Assessment of the Method

Compounds with known α_2-adrenoceptor blocking activity antagonize clonidine-induced sleep in chicks dose dependently. Yohimbine is one of the most active compounds. However, also other centrally active compounds of which their main effect is not α_2-blockade reduce clonidine-induced sleeping time. One of the most potent is apomorphine acting on dopamine D_2-receptors. Therefore, the clonidine-induced sleeping test in chicks cannot be regarded as highly specific for α_2-adrenoceptors.

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Activity at β_1- and β_2-Adrenoreceptors in the Rat

Purpose and Rationale

The relative potency of catecholamines as stimulants of β-adrenoreceptor-mediated responses varies in different tissues indicating the existence of two subtypes of β-receptors (β_1 and β_2) (Lands et al. 1967a, b). β-Adrenoreceptors in the heart have been classified as being of the β_1-subtype. β-Adrenoreceptors in the uterus, diaphragm, bronchioles, and small intestine have been classified as being of the β_2-subtype, since in these tissues, epinephrine is more potent than norepinephrine. These observations led to the development of selective agonists and antagonists. Isolated organs (see below) having predominantly one receptor subtype, such as the isolated heart and the isolated atrium for β_1, and the isolated uterus or the isolated tracheal chain for β_2, are used to test compounds for selective activity. Assessing both activities in the same animal in vivo results in the advantage that pharmacokinetic and metabolic influences of the drug being tested are the same for both parameters.

Procedure

Female Sprague–Dawley rats (200–220 g) are anesthetized with 60 mg/kg pentobarbital i.p. prior to pithing (Gillespie and Muir 1967). The animals are artificially respired with room air using a Harvard small animal ventilator (90 strokes/min at a pressure of 7 cm H_2O). Body temperature is maintained by placing the animals on a heated operating table. The left carotid artery is cannulated for continuous monitoring of blood pressure via a Statham P23 ID pressure transducer. The blood pressure signal is used to trigger an instantaneous rate meter for continuous monitoring of heart rate. A femoral vein is cannulated for intravenous administration of drugs.

A midline incision is made to expose one horn of the uterus. The ovarian artery is cut, tied, and one horn dissected free from the ovary leaving the myometrial blood supply intact. A cotton thread is attached to the free end of the uterine horn, passed through a glass-jacketed organ bath and connected to an isometric (Pioden UF1) transducer for measurement of spontaneous contractions. A cannula is inserted into the peritoneal cavity for administration of drugs by the i.p. route. The organ bath is positioned such that it surrounds the uterine horn without touching it. The tissue is perifused with Krebs–Henseleit solution being gassed with 95 % O_2/5 % CO_2 and maintained at 37 °C. A resting tension of 0.2 g is applied to the tissue, which is allowed to stabilize until spontaneous contractions are constant over a period of 5–10 min. All recordings are made on a polygraph.

Evaluation of Agonists

Dose–response curves after i.v. injection are established for isoprenaline (nonselective between β_1- and β_2-adrenoreceptors), salbutamol (selective for β_2-adrenoreceptors), and noradrenaline (selective for β_1-adrenoreceptors) in increasing heart rate (beats/min) and decreasing the height of uterine contraction (calculated as percentage of the original amplitude). Animals given noradrenaline are pretreated with phenoxybenzamine (3.3 mmol/kg i.v.) in order to antagonize irreversibly the α-adrenoreceptors. Agonist dose–response curves ($n > 4$) on heart

rate and uterine relaxation are carried out by assessing the activity of at least three doses of each agonist. New synthetic compounds can be tested after intraperitoneal administration additionally.

Evaluation of Antagonists

The ability of a nonselective β-blocker, such as propranolol (1 mmol/kg i.v.); a β_1-selective β-blocker, such as atenolol; and a β_2-selective β-blocker to inhibit responses to isoprenaline on both heart rate and uterine relaxation is assessed by comparing the log-linear portion of the dose–response curve to isoprenaline in the absence and in the presence of the β-adrenoreceptor antagonist in the same animal. Dose ratios for each antagonist are calculated.

Critical Assessment of the Method

The method described by Piercy (1988a, b) has the advantage to measure both agonistic and antagonistic activity and to differentiate between effects on β_1- and β_2-adrenoreceptors. Compared to tests in isolated organs, in vivo activity can be determined after intraperitoneal or intraduodenal administration.

Modifications of the Method

Härtfelder et al. (1958) studied the influence of various agents on the contractions of electrically stimulated **isolated uteri of rabbits and guinea pigs**.

Nathason (1985) evaluated the activity of beta-blockers to inhibit the cardioacceleratory effect of systemically administered isoproterenol in **unanesthetized, restrained albino rabbits** together with the effect on membrane-bound adenylate cyclase in homogenized ciliary process villi in order to find compounds selectively lowering intraocular pressure.

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β_1- and β_2-Sympatholytic Activity in Dogs

Purpose and Rationale

Intravenous administration of isoprenaline (isoproterenol) stimulates β_1-receptors of the heart which can be detected as an increase in contractility (dp/dt max). Intra-arterial injection of isoprenaline stimulates β_2-receptors of peripheral blood vessels leading to an increased peripheral blood flow.

Therefore, a β_1- or β_2-blocking activity of a compound is revealed by the inhibition of the effects of isoprenaline. The following tests are used to evaluate β-blocking activity of drugs. A β-blocker screening is done in anesthetized dogs (a); in addition, the test allows a differentiation between β_1- and β_2-receptor activity and the determination of ED_{50} values (b).

Procedure

Male or female Beagle dogs weighing about 20 kg are used. Animals are premedicated with 1 g Inactin (i.v.) and anesthetized by intravenous administration of 20 mg/kg chloralose and 250 mg/kg urethane. In addition, they receive a subcutaneous injection of 2 mg/kg morphine 1 h after the start of anesthesia. Animals are heparinized. Respiration is maintained through a tracheal tube using a positive pressure respirator. End-expiratory CO_2 content is measured continuously; respiratory rate and depth of respiration are adjusted to 4.5–6 vol.% end-expiratory CO_2. For administration of isoprenaline, a peripheral vein is cannulated.

Preparation for Hemodynamic Measurements

For recording of peripheral systolic and diastolic blood pressure, a cannula inserted into a femoral artery is connected to a pressure transducer (Statham P23 DB). For determination of LVP, a Millar microtip catheter (PC 350) is inserted via the left arteria carotis communis. LVEDP is measured from a high-sensitivity scale. From the pressure curve, dp/dt max is differentiated and heart rate is counted. Peripheral blood flow in the femoral artery is measured with an electromagnetic flow probe.

Screening for β-Blocking Effects in Anesthetized Dogs

Following a steady-state period of 30–60 min, isoprenaline is administered intravenously two to three times to the anesthetized animal and hemodynamic parameters are recorded (control values = 100 %). Then, the test substance is injected intravenously at cumulative doses (final concentrations of 0.01, 0.05 and 0.15 mg/kg). For each dose, 10-min "drug effects" are monitored by measuring hemodynamic parameters. Then the effect of isoprenaline is tested again (three times).

In other experiments, a single dose of the drug is administered to determine the duration of action.

If a test compound does not show an inhibitory influence on isoprenaline effects, a second test compound is administered.

All hemodynamic parameters are registered continuously during the whole experiment.

Testing for β_1- and β_2-Blocking Effects: Determination of ED_{50}

Following a steady-state period of 30–60 min, isoprenaline is administered for i.v. administration (β_1-test) twice at a dose of 0.5 μ g/kg and for intra-arterial administration (β_2-test) twice at a dose of 0.05 μ g/kg. Hemodynamic parameters are recorded (control values = 100 %). Then, the test substance is injected intravenously at cumulative doses. Consecutively increasing doses are given at 15-min intervals. For each dose, 10-min "drug effects" are monitored by measuring hemodynamic parameters. Thereafter, isoprenaline is given intravenously and 5 min later intra-arterially.

All hemodynamic parameters are registered continuously during the whole experiment.

Characteristics:

- Blood pressure
  - Systolic, BPs
  - Diastolic, BPd
- Heart rate
- Left ventricular pressure, LVP
- Left ventricular end-diastolic pressure, LVEDP
- dp/dt max
- Peripheral flow, A. femoralis
- ECG, lead II

Standard compounds:

- Propranolol HCl
- Practolol
- Metoprolol tartrate

Evaluation

β_1-Receptor antagonism is measured as a decrease in contractility (dp/dt max).

Inhibition of the isoprenaline-induced elevation of heart rate is considered as an indicator for nonselective β-blockade. For cardioselective β-receptor blockers, the increase in dp/dt max is inhibited with lower doses of test drug than the rise in heart rate.

β_2-receptor blockade by a test drug is measured as inhibition of the isoprenaline-induced increase in peripheral blood flow.

The different hemodynamic parameters are determined.

Percent inhibition of the isoprenaline-induced effects by a test compound is calculated and compared to the isoprenaline effects before drug administration (=100 %).

ED_{50} values for $\beta 1$- and $\beta 2$-antagonism are calculated by log-probit analyses. ED_{50} is defined as the dose of drug leading to a 50 % inhibition of the isoprenaline effects.

An $ED_{50}\ \beta_1/ED_{50}\ \beta_2$-ratio of <1 indicates that a β-blocking agent predominantly influences β_1-receptors (cardioselectivity).

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Intrinsic β-Sympathomimetic Activity in Reserpine-Pretreated Dogs

Purpose and Rationale

β-Blocking agents can be classified as:

- β-Blocking agents with intrinsic sympathomimetic activity (ISA)
- β-Blocking agents with membrane stabilizing activity (MSA)
- β-Blocking agents with organ selectivity (high affinity to heart β_1-receptors)

In the following procedure with reserpine-pretreated dogs, β-blocking agents with intrinsic sympathomimetic activity can be identified. Reserpine administration 24 h before the start of the experiment leads to a depletion of catecholamine depots. Thus, it is possible to differentiate between indirectly acting sympathomimetics such as tyramine and directly acting ones such as noradrenaline.

This test is used to identify β-blocking drugs with intrinsic sympathomimetic activity.

Procedure

Male or female Beagle dogs weighing about 15 kg are used. Twenty-four h before the test, dogs receive an intramuscular injection of 0.3 mg/kg reserpine. At the day of the experiment, the animals are anesthetized by intravenous administration of 10–20 mg/kg sodium pentobarbital. Respiration through a tracheal tube using a positive pressure respirator is controlled by measuring end-expiratory CO_2 concentrations (4–5 vol.%).

Preparation for Hemodynamic Measurements

For recording of peripheral systolic and diastolic blood pressure, a femoral artery is cannulated and connected to a pressure transducer (Statham P23 DB). For determination of LVP, a Millar microtip catheter (PC 350) is inserted into the left ventricle via the left common carotid artery. LVEDP is measured from a high-sensitivity scale. From the pressure curve, dp/dt max is differentiated and heart rate is counted.

Experimental Course

The test substance is administered by continuous intravenous infusion of 0.02 mg/kg (1 ml/min) until a cumulative dose of 3 mg/kg is achieved (within approximately 150 min). Thereafter, the velocity of infusion is doubled (0.04 mg/kg, 2 ml/min). The test is finished when a cumulative dose of 7 mg/kg is achieved (after a total time of approximately 250 min).

Hemodynamic parameters are registered continuously during the entire experiment.

Characteristics:

- Blood pressure
  - Systolic blood pressure
  - Diastolic blood pressure
- Left ventricular pressure, LVP
- Left ventricular end-diastolic pressure, LVED
- d*p*/d*t* max
- Heart rate, HR

Evaluation

The different hemodynamic parameters are determined. As a measure for intrinsic sympathomimetic activity (ISA), the increase in d*p*/d*t* max and in heart rate are evaluated. Absolute and relative differences of these parameters in drug-treated animals are compared to vehicle control values.

Statistical evaluations are performed by means of the Student's *t*-test if $n > 4$.

Scores are allotted relative to the efficacy of standard compounds for intensity as well as for duration of the effect.

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Cat Nictitating Membrane Preparation (Ganglion-Blocking Activity)

Purpose and Rationale

Nicotinic acetylcholine receptors are involved in the ganglionic neurotransmission. Various subtypes are described for nicotinic acetylcholine receptors (Sargent 1995; McGehee and Role 1995; Karlin and Akabas 1995; Alexander et al. 2001).

The nictitating membrane of the cat has been used extensively in pharmacological studies to evaluate ganglion-blocking activity because of the ease with which its movements can be recorded, because of the simplicity of its innervation (the purely adrenergic fibers have their cell bodies in the easily accessible superior cervical ganglion of the same site), and because its blood supply (via the external carotid artery) is accessible for intra-arterial injections. Preganglionic and postganglionic stimulation allow the interpretation of the mode of action of vasoactive drugs.

Procedure

The animal is anesthetized with 35 mg/kg sodium pentobarbital i.p. Tracheostomy is performed and a tracheal cannula is inserted. On one side, the sympathetic nerve is exposed, separated from the vagus nerve, and prepared in order to place electrodes for preganglionic and postganglionic stimulation. Preferably, the vagus nerve at this site is severed at the central end. The head of the animal is fixed in a head holder to prevent head movements. A linear transducer is fixed at the mid of the border of the nictitating membrane allowing the registration of the contractions on a polygraph. Preganglionic and postganglionic stimuli are exerted by a square wave stimulator, with a pulse width of 0.3–0.5 ms, an amplitude of 1–3 V, and a frequency of 20/min. The amplitude and pulse width varies from animal to animal. The sympathetic nerve is stimulated before and after the administration of the compound, and the changes in the contraction of the nictitating membrane are noted. Furthermore, the response of the nictitating membrane to exogenous adrenaline is registered.

Evaluation

The decrease of the response after drug application is expressed as percentage of the control

before drug. Ganglionic blockers decrease the response to preganglionic stimulation but have no influence on postganglionic stimulation or exogenous adrenaline. Neuronal blockers decrease the response to both preganglionic and postganglionic stimulation but do not affect the response to exogenous adrenaline which may even be enhanced. α-receptor blockers decrease the response to both preganglionic and postganglionic stimulation as well as decrease the effect of exogenous adrenaline. Catecholamine uptake inhibitors increase the response to both preganglionic and postganglionic stimulation as well as enhance the response to exogenous adrenaline.

Critical Assessment of the Method

The nictitating membrane preparation has been widely used for differentiation of cardiovascular effects. Since the use of higher animals such as cats has been limited to a great extent, this model is now being used only exceptionally.

As alternative, the contraction of the inferior eyelid of anesthetized **rats** after preganglionic electrical stimulation of the superior cervical ganglion has been recommended (Gertner 1956; Steinbrecher and Schmid-Wand 1986). In the modification used by Steinbrecher and Schmid-Wand (1986), the method is suitable for testing compounds with potential adrenergic and antiadrenergic activity but not for testing ganglion-blocking activities.

Male Sprague–Dawley rats are anesthetized with 100 mg/kg thiobutabarbital i.p. and kept on a heated operation table at a rectal temperature of 37 °C. One femoral vein is cannulated and filled with 4 % heparin solution. One femoral artery is cannulated for registration of blood pressure. Tracheotomy is performed and a polyethylene catheter of 5 cm length inserted. The head of the animal is fixed carefully. The vibrissae at the lower eyelid on the right side are cut, a thread attached at the margin of this eyelid and attached to a strain gauge. To immobilize the musculature of the face, the mouth of the animal is sutured and the head support attached. The right sympathetic nerve is exposed, separated from the vagus nerve, and prepared in order to place electrodes for preganglionic stimulation. For calibration, stimulation is performed twice with an interval until contraction is back to baseline. Furthermore, a dose of 0.001 mg/kg adrenaline is given as bolus injection. Eyelid contraction and blood pressure increase are recorded. Then the putative adrenergic blocker or the standard 1.0 mg/mg phentolamine is injected intravenously. Eyelid contraction after electrical stimulation or after adrenaline is reduced dose dependently.

Modifications of the Method

Quilliam and Shand (1964) assessed the selectivity of drugs by comparing the effects on ganglionic transmission and on the pre- and postganglionic nerves in the isolated superior cervical ganglion preparation of the rat.

Langer and Trendelenburg (1969) performed experiments with normal nictitating membranes of pithed cats as well as with isolated normal nictitating membranes.

Koss and Hey (1992) used frequency-dependent nictitating membrane responses by sympathetic nerve stimulation in anesthetized cats to determine the potential role of prejunctional histamine H_3 receptors.

Gurtu et al. (1992) used contractions of the cat nictitating membrane to explore the effects of calcium channel blockers on neurotransmission in vivo, by comparing the effects of verapamil and nifedipine on contractions of nictitating membrane following either electrical stimulation of the superior cervical ganglion or intravenous injection of phenylephrine.

Koss (1992) compared the peripheral and central nervous system sympatholytic actions of prazosin using the cat nictitating membrane. Submaximal contractions of the nictitating membranes were evoked by electrical stimulation of the preganglionic cervical sympathetic nerve

trunk and by stimulation of the posterior hypothalamus in anesthetized cats.

Badio et al. (1996) evaluated spiropyrrolizidines, a new structural class of blockers of nicotinic receptor channels with selectivity for ganglionic-type receptors in rat pheochromocytoma PC12 cells (with an $\alpha_3\beta_{4(5)}$-nicotinic receptor) and human medulloblastoma TE671 cells (with an $\alpha_1\beta_1\gamma\delta$-nicotinic receptor).

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Assessment of Ganglion-Blocking Activity in the Isolated Bovine Retractor Penis Muscle

Purpose and Rationale

The use of the bovine retractor penis muscle for the assessment of ganglion-blocking activity of neuromuscular blocking drugs has been recommended by Alaranta et al. (1990) and Klinge et al. (1993). Klinge and Sjöstrand (1974) performed not only extensive studies on the physiology and pharmacology of the retractor penis in the bull but also discussed the various hypotheses on inhibitory and excitatory innervation of this muscle, which is present in many vertebrates such as horses, cats, dogs, and rats, but not in men and rabbits. They also found that the effects on the isolated retractor penis muscle and on penile arteries are rather similar. The excitatory innervation was found to be predominantly α-adrenergic (Klinge et al. 1970; Klinge and Sjöstrand 1977), whereas other transmitters such as histamine and bradykinin were effective only in some species. Relaxation of the isolated retractor penis muscle could be elicited by nicotine and other nicotinic agonists (Klinge et al. 1988). In the studies on ganglion-blocking activity, strips of the retractor penis muscle are precontracted by 5-hydroxytryptamine. Relaxation induced by nicotine is antagonized by ganglion blockers.

Procedure

Retractor penis muscles are obtained from bulls of different breeds weighing 250–500 kg. Samples are dissected 10–30 cm distal to the points where the paired muscle bundles pass the anal orifice. Immediately after slaughter, the samples are freed from fat and other surrounding tissue and placed into Tyrode solution at 2–4 °C. Strips, 15–25 mm in length and 2–3 mm wide, are prepared and mounted in 20-ml organ baths containing Tyrode solution at 35 °C aerated with 95 % O_2 and 5 % CO_2. An equilibrium time of 2–4 h is allowed. During the equilibrium period, washing is performed at about 60-min intervals. Changes in tension are recorded by means of Grass FT03 force displacement transducers coupled to a polygraph.

A high-enough tone for studying the nicotine-induced relaxation, usually 8–15 g, is generated by adding 5-HP in a concentration between 0.1 and 6 μM to the organ bath. Washing is performed 2 min after application of nicotine; 60–80 min later, the tone is again raised and the application of nicotine is repeated. The effect of a neuromuscular blocking drug is studied only if the relaxations caused by nicotine in two consecutive controls are equal in size.

Evaluation

The blocking activity of a certain concentration of a drug is expressed as % reduction in the relaxation of the muscle strip, according to the following equation:

$$\frac{A - B}{A} \times 100$$

where A is the size of the control relaxation in millimeters and B is the size of the relaxation of the blocking drug. In order to construct regression lines, the activity of four or five dose levels from the assumed linear part of the concentration–effect curve is studied. The activity of each dose level is studied in at least five strips obtained from different animals. IC_{50} values are calculated from the regression lines. The parallelism of the regression lines is tested by covariance analysis.

Critical Assessment of the Method

Molar potency ratios of known ganglion-blocking agents obtained with this method were

compared with the results of other methods, such as inhibition of contraction of cat nictitating membrane evoked by preganglionic sympathetic stimulation (Bowman and Webb 1972; see section "Cat Nictitating Membrane Preparation" in this chapter), inhibition of nicotine-induced contraction of the isolated guinea pig ileum (Feldberg 1951), inhibition of contraction of guinea pig vas deferens evoked by preganglionic stimulation of the hypogastric nerve in vitro (Birmingham and Hussain 1980), depression of postganglionic action potentials evoked by preganglionic stimulation of the superior cervical ganglion of the rat in vitro (Quilliam and Shand 1964), and induction of mydriasis in mouse by blocking the ciliary ganglion (Edge 1953). A fair but not a complete agreement between the results obtained with various methods was found.

Modifications of the Method

Gillespie and Sheng (1990) studied the effects of pyrogallol and hydroquinone on the response to nonadrenergic, non-cholinergic nerve stimulation in the rat anococcygeus and the bovine retractor penis muscles.

Parkkisenniemi and Klinge (1996) used samples of retractor penis muscles and penile arteries from bulls for functional characterization of endothelin receptors.

La et al. (1997) studied the inhibition of nitrergic nerve-induced relaxations in rat anococcygeus and bovine retractor penis muscles by hydroxycobalamin.

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Angiotensin II Antagonism

Purpose and Rationale

Angiotensin II antagonists can be tested in rats after elimination of cardiovascular reflexes by vagotomy and ganglionic blockade. Several angiotensin II antagonists possess intrinsic agonistic activity. This can be tested by injection of various doses to the vagotomized, ganglion-blocked animal. The antagonistic activity of the angiotensin II antagonist can be evaluated by antagonism against graded doses of angiotensin II. The duration of activity can be tested during continuous infusion of angiotensin II.

Procedure

Male Sprague–Dawley rats weighing about 300 g are used. They are anesthetized with 60 mg/kg sodium pentobarbital i.v. One carotid artery is cannulated and connected with a Statham P23 DB transducer. Blood pressure is recorded on a polygraph. Both jugular veins are cannulated for application of test compounds and for infusion. Both vagal nerves are cut 3 mm dorsal of the larynx. For ganglionic blockade, 10 mg/kg pentolinium tartrate is injected intravenously. At least five animals are used for evaluation of one test drug.

Intrinsic Agonistic Activity

After the blood pressure has reached a constant value, doses of 1, 2, 4, and 16 μg/kg of the test compound are injected via the jugular vein. Blood pressure is recorded.

Antagonistic Activity

In 10-min intervals, doses of 0.5, 1.0, and 2.0 μg/kg angiotensin II are injected to establish dose–response curves. After 10 min, continuous infusion is started of the potential angiotensin II blocker in a dosage of 10 μg/kg/0.1 ml/min. Ten minutes after beginning of the infusion, again doses of 0.5, 1.0, and 2.0 μg/kg angiotensin II are injected.

Duration of Activity

In this setup, angiotensin II is administered as continuous infusion at a dosage of 1 μg/kg/0.02 ml/min. When blood pressure has reached an elevated steady-state level, 0.1 mg/kg of the angiotensin II antagonist is administered.

Intensity and duration of the fall of blood pressure are recorded.

Evaluation

Intrinsic Agonistic Activity

An increase of blood pressure indicates the intrinsic agonistic activity.

Antagonistic Activity

Increases of blood pressure after graduated doses of angiotensin II during the infusion is expressed as percentage of the increase before infusion. The results are compared with known angiotensin II antagonists.

Critical Assessment of the Method

In this test, not only the potency and duration of activity but also the intrinsic agonistic activity of an angiotensin II antagonist can be tested.

Modifications of the Method

Various other pharmacological models have been used to test angiotensin II antagonists:

Blood pressure in conscious unrestrained rats with chronically implanted catheters with normal

blood pressure, spontaneous hypertension, and chronic renal hypertension (Vogel et al. 1976; Chiu et al. 1989; Brooks et al. 1992; Aiyar et al. 1995; Deprez et al. 1995; Gabel et al. 1995; Hilditch et al. 1995; Keiser et al. 1995; Nagura et al. 1995; Nozawa et al. 1997; Renzetti et al. 1995; Wong et al. 1995; Junggren et al. 1996)

Blood pressure in conscious spontaneously hypertensive and in anesthetized ganglion-blocked rats (Olins et al. 1993)

Blood pressure in pithed and in conscious renovascular hypertensive rats (Criscione et al. 1993; Wienen et al. 1993; Deprez et al. 1995; Kivlighn et al. 1995a; Kushida et al. 1995)

Blood pressure in rats after intracerebroventricularly injected angiotensin II (Vogel et al. 1976; Batt et al. 1988)

Blood pressure in conscious angiotensin I-infused and renin-dependent **hypertensive dogs** (Brooks et al. 1992; Cazaubon et al. 1993; Aiyar et al. 1995; Deprez et al. 1995; Gabel et al. 1995; Keiser et al. 1995; Wong et al. 1995)

Blood pressure and heart rate in conscious sodium-depleted and sodium-repleted **cynomolgus monkeys** (Lacour et al. 1993; Cazaubon et al. 1993; Keiser et al. 1995)

Angiotensin II-induced pressor responses in **marmosets** (Nagura et al. 1995)

Blood pressure and heart rate in conscious **rhesus monkeys** and anesthetized **chimpanzees** (Gabel et al. 1995; Kivlighn et al. 1995b; Kivlighn et al. 1995c)

Inhibition of angiotensin II-induced contraction in isolated **aorta** rings or strips from **rabbits** (Chiu et al. 1989; Chui et al. 1990; Criscione et al. 1993; Cazaubon et al. 1993; Olins et al. 1993; Wienen et al. 1993; Aiyar et al. 1995; Caussade et al. 1995; Hilditch et al. 1995; Keiser et al. 1995; Kushida et al. 1995; Nagura et al. 1995; Renzetti et al. 1995; Wong et al. 1995), from **rats** (Nozawa et al. 1997), from **neonatal rats** (Keiser et al. 1993), and from **guinea pigs** (Mizuno et al. 1995)

Inhibition of angiotensin II-induced contraction in isolated rat pulmonary artery (Chang et al. 1995)

Antagonism against angiotensin II in isolated strips of rabbit aorta, rabbit jugular vein, rabbit pulmonary artery, rat portal vein, rat stomach, rat urinary bladder, human urinary bladder, human colon, and human ileum (Rhaleb et al. 1991)

Contractions of **guinea pig ileum** in situ (Khairallah and Page 1961)

Antagonism against angiotensin II in the **isolated rat uterus** (Wahhab et al. 1993)

Contractile force and prostaglandin E synthesis in electrically stimulated **rabbit isolated vas deferens** (Trachte et al. 1990)

Antagonism against angiotensin II-induced aldosterone release in **bovine adrenal glomerulosa cells** (Criscione et al. 1993) and in rat dispersed adrenal capsular cells (Chang et al. 1995)

Antagonism against angiotensin II-induced inhibition of guanylate cyclase activity in the **rat pheochromocytoma cell line** PC12W (Brechler et al. 1993)

Brooks et al. (1995) compared the cardiovascular and renal effects of an angiotensin II receptor antagonist and captopril in **rats with chronic renal failure** induced by 5/6 nephrectomy. Under sodium pentobarbital anesthesia, the right kidney was removed and approximately two thirds of the left kidney was infarcted by ligating two or three branches of the left renal artery.

Kim et al. (1997) studied the effects of an angiotensin AT1 receptor antagonist on volume overload-induced cardiac gene expression in rats. An abdominal aorta-caval shunt was prepared in 9-week-old male Wistar rats under sodium pentobarbital anesthesia. The vena cava and the abdominal aorta were exposed by opening the abdominal cavity via a midline incision. The aorta was punctured at the union of the segment two thirds caudal to the renal artery and one third cephalic to the aortic bifurcation with a 18-gauge disposable needle. The needle was advanced into the aorta, perforating its adjacent wall and penetrating the vena cava. After the aorta was clamped, the needle was withdrawn, and a drop of cyanoacrylate glue was used to seal the aortic puncture point. The patency of the shunt was verified visually by swelling of the vena cava and mixing of arterial and venous blood. The rats were treated either with vehicle

or the angiotensin antagonist. Four days after the preparation of the AC shunt, 24-h urine volume, electrolytes, and aldosterone were measured. Six days after the AC shunt, blood was collected by puncture of a tail vein and plasma renin activity and aldosterone were measured. Seven days after AC shunt, hemodynamic studies were performed in pentobarbital anesthesia. Afterwards, the heart was rapidly excised and the left and right atria and ventricles were separated and frozen in liquid nitrogen for the extraction and measurement of cardiac tissue RNA.

Shibasaki et al. (1997) tested the effect on the renin–angiotensin–aldosterone system in **conscious rats** after cannulation of the abdominal aorta under anesthesia 3–4 days before the experiment. After oral dosing of the angiotensin II receptor antagonist, blood samples were withdrawn and plasma renin and aldosterone determined by radioimmunoassay.

Similar to the effects of ACE inhibitors, lifespan of hypertensive rats could be doubled by long-term treatment with an angiotensin II type 1 receptor blocker (Linz et al. 2000).

Ledingham and Laverty (1996) treated **genetically hypertensive New Zealand rats** with a specific AT_1 receptor antagonist via osmotic minipumps for several weeks and measured the effects on blood pressure, cardiac hypertrophy, and the structure of resistance arteries.

Transgenic animals were recommended for further studies to influence the human renin–angiotensin system (Müller et al. 1995; Wagner et al. 1996; Bohlender et al. 1996).

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ACE Inhibition Measured In Vivo in the Rat

Purpose and Rationale

The angiotensin-converting enzyme (ACE) is responsible for the cleavage of angiotensin I to the active angiotensin II. ACE is responsible for the degradation of the active peptide bradykinin to inactive products. ACE activity can therefore be measured in two ways: activity of the newly formed angiotensin II and diminution of the activity of bradykinin. ACE inhibition results in decreased activity of the precursor angiotensin I and potentiation of the bradykinin effect. The cardiovascular system is sensitive to both peptides, reacting with an increase of blood pressure to angiotensin II and with a decrease to bradykinin. These reactions can be used for quantitative determination of ACE-inhibiting activity.

Procedure

Male Sprague–Dawley rats weighing 300–400 g are used. The animals are anesthetized by i.p. injection of 70 mg/hg pentobarbital. After intubation of the trachea, they are artificially respirated with 30 strokes/min and a stroke volume of 6–8 ml. The right carotid artery is cannulated and blood pressure registered with a Statham element (P23 DB) and a polygraph. One jugular vein is cannulated for i.v. injections. After laparotomy a catheter is inserted into the duodenum for enteral administration and the wound closed again. Blood pressure is stabilized 30 % below the normal level by i.m. injection of 5 mg/kg pentolinium. In order to prevent excessive mucus production in the bronchial system, 40 μg/kg atropine sulfate is injected intramuscularly.

Inhibition of Angiotensin I Cleavage

After stabilization of blood pressure, 310 ng/kg angiotensin I is injected intravenously in 0.1 ml saline. The injection is repeated in 5-min intervals until an identical pressure reaction occurs. The test compounds are administered at doses of 1 and 10 mg/kg intravenously or 25 mg/kg intraduodenally. Three minutes after i.v. injection or 10 min after i.d. administration, again 310 ng/kg angiotensin I is injected. Standards are ramipril, enalapril, or captopril.

Potentiation of Bradykinin-Induced Vasodepression

A low dose of bradykinin has to be chosen in order to visualize the bradykinin potentiation. One μg/kg, eventually 3 μg/kg bradykinin are injected intravenously at 5-min intervals until a stable reaction is achieved. Three minutes after i.v. injection or 10 min after intraduodenal administration of the test substance, the bradykinin injection is repeated.

Evaluation

Inhibition of Angiotensin I Cleavage

The diminution of the pressure reaction to angiotensin I after administration of a potential ACE inhibitor is the parameter for the activity of the new compound. The inhibition is calculated as percent of controls. Using various doses of the ACE inhibitor, dose–response curves can be established and ID_{50} values be calculated.

Potentiation of Bradykinin-Induced Vasodepression

Potentiation of bradykinin-induced vasodepression is expressed as percentage of controls. Using various doses of the test compound and the standard, dose–response curves can be established and potency ratios calculated.

Critical Assessment of the Method

Both parameters, inhibition of angiotensin I response and potentiation of bradykinin-induced vasodepression, have been proven as reliable parameters for evaluation of ACE inhibitors.

Modifications of the Method

Natoff et al. (1981) used the ratio of responses to angiotensin I and angiotensin II in spontaneously hypertensive rats, either pithed or anesthetized with urethane, to determine the degree and the duration of effect of captopril.

Blood levels of angiotensin II can also be measured by radioimmunoassay.

Several studies in rats showed the beneficial effects of prolonged treatment with ACE inhibitors. Postoperative mortality in rats with left ventricular hypertrophy and myocardial infarction was decreased by ACE inhibition (Linz et al. 1996).

Inhibition of angiotensin I-induced pressure response by administration of ACE inhibitors can be measured not only in anesthetized rats but also in anesthetized dogs, conscious rats, and conscious dogs (Becker et al. 1984).

Lifelong ACE inhibition doubles the life-span of hypertensive rats not only if the treatment is started at the age of 1 month (Linz et al. 1997), but ramipril also increases survival in old spontaneously hypertensive rats if treatment is started at the age of 15 months (Linz et al. 1999).

Panzenbeck et al. (1995) reported that captopril-induced hypotension is inhibited by the bradykinin blocker HOE 140 in Na^+-depleted **marmosets**.

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Evaluation of Renin Inhibitors in Dogs

Purpose and Rationale

Highly specific inhibitors of the enzyme renin are considered to be potential antihypertensive agents. These agents cause a fall in blood pressure of sodium-deficient dogs and decrease plasma renin activity as well as angiotensin II level.

Procedure

Animal Experiment

Adult mongrel dogs (8–14 kg) of either sex are given water ad libitum and maintained on a low sodium diet for 1–2 weeks before the experiment. A single intramuscular injection of 5 mg/kg furosemide is given 48 h before the experiment. On the day of the experiment, the dogs are anesthetized with sodium pentobarbital (30 mg/kg i.v.) and a cuffed endotracheal tube is positioned to allow artificial respiration. To measure arterial blood pressure, a femoral artery is catheterized with polyethylene tubing. The right and left femoral veins are catheterized for drug administration and delivery of a maintenance infusion of sodium pentobarbital (5 mg/kg/h). Blood pressure is measured directly through the catheter, which is connected to a Gould–Statham pressure transducer. Blood samples are collected from the arterial catheter.

Increasing doses of the potential renin inhibitor are infused over 30 min followed by a 30-min recovery period. Immediately after the last recovery period, the dogs are given an i.v. infusion of the angiotensin receptor antagonist saralasin (20 µg/kg/min) for 30 min. For measurement of plasma renin activity and angiotensin II levels, the dogs are infused over a period of 30 min with the test compound and blood is withdrawn at 0, 15, 30, 60, 90, 120, 180, and 240 min after the start of the infusion. After the final blood drawing, 20 µg/kg/min saralasin is infused for 30 min.

Analytical Procedures

The antibody-trapping method is preferred to measure plasma renin activity (PRA). In this procedure, PRA is determined at pH 7.4 by RIA quantification of angiotensin I (ANG I) generated and then trapped by excess anti-ANG I antibody (Poulsen and Jørgensen 1973; Nussberger et al. 1987). In tubes coated with rabbit anti-ANG I antibody (Gamma Coat™ ^{125}I plasma renin activity RIA kit; Baxter Travenol Diagnostics) and incubated in an ice-water bath, 75 µl plasma is mixed with 7 µl 3 M TRIS base buffer (pH 7.2) containing 200 mM EDTA and 3 µl 0.2 M TRIS base (pH 7.5) containing 3 g/L human serum albumin (fraction V, Sigma). Tubes are vortexed and incubated at 37 °C for 60 min. The incubation is terminated by placing the tubes in an ice-water bath. Next, 75 µl of the TRIS albumin buffer are added, followed by 1 ml phosphate RIA buffer (Gamma Coat™) containing 15,000 cpm of ^{125}I ANG I. Standard ANG I (0.2–50 ng/ml) is also incubated at 37 °C for 60 min with 10 10 µl mu;l TRIS/albumin buffer. In an ice-water bath, low renin plasma (75 µl) is added to the standards before the addition of a 1 ml tracer solution. Samples and standards are incubated for 24 h at 4 °C. Tubes are then aspirated and counted in a gamma counter.

Levels of immunoreactive angiotensin II (ir-ANG II) are measured using a procedure described by Nussberger et al. (1985). Two to three ml of whole blood is collected in prechilled glass tubes containing 125 µl of the following "inhibitor" solution: 2 % ethanol, 25 mM

phenanthroline, 125 mM EDTA, 0.5 mM pepstatin A, 0.1 mM captopril, 2 g/l neomycin sulfate, and 0.1 mM of the renin inhibitor CGP 38560. The tubes are then centrifuged and the plasma quickly frozen in liquid nitrogen and stored at −70 °C. For extraction of angiotensin peptides, Bond Elut cartridges (Bond Elut pH) containing 100 mg phenylsilica are used, along with a Vac Elut SPS 24 vacuum manifold (Analytichem; Harbor City, CA). Each cartridge is preconditioned with 1.0 ml methanol (HPLC grade) followed by 1.0 ml of water (HPLC grade) at a vacuum pressure of 5 mmHg. One milliliter of the thawed sample is then applied to the cartridge and washed with 3 ml HPLC grade water. The angiotensin peptides retained at the columns are eluted with 0.5 ml methanol (HPLC grade, vacuum pressure less than 5 mmHg) into polypropylene tubes coated with a buffer containing 0.2 M TRIS, 0.02 % NaN_3, and 2.5 mg/ml fatty acid-free bovine serum albumin (pH 7.4 with glacial acetic acid). The methanol is evaporated at 40 °C and ir-ANG II measured using an antibody (IgG Corp., Nashville, TN) with greater than 1,000-fold selectivity for ANG II.

Evaluation

All data are expressed as mean ± SEM. The hypotensive responses after various doses of the renin antagonist are compared with the inhibition of plasma renin activity and the decrease of immunoreactive angiotensin II.

Critical Assessment of the Method

The antibody-trapping method, reported here, gives a better correlation with the blood pressure-lowering effect in dogs than the conventional method based on RIA for generated ANG I (Palmer et al. 1993).

Modifications of the Method

Pals et al. (1990) described a rat model for evaluating inhibitors of human renin using anesthetized, nephrectomized, ganglion-blocked rats. The blood pressure rise induced by sustained infusion of renin was dose dependently decreased by a renin inhibitor.

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Evaluation of Renin Inhibitors in Monkeys

Purpose and Rationale

The renin–angiotensin system as the main regulator of blood pressure can be influenced in several ways. One approach involves the inhibition of renin. Renin is an aspartyl protease that hydrolyzes angiotensinogen to release the decapeptide angiotensin I, which is subsequently converted to angiotensin II by angiotensin-converting enzyme.

Sequencing of renin and angiotensinogen from various species revealed marked species differences for both the enzyme and the substrate. Inhibitors developed for human renin show a high specificity for primate renin and show only weak inhibition of renin from subprimate species. This means that the most common laboratory animals, such as rats and dogs, are not suitable for the in vivo evaluation of renin inhibitors. The marmoset was chosen by Wood et al. (1985, 1989a) as a primate model.

Procedure

Marmosets (*Callithrix jacchus*) of both sexes weighing between 300 and 400 g are fed a pellet diet supplemented with fruit. Two days prior to the experiment, the animals are anesthetized and catheters are implanted in a femoral artery for measurement of blood pressure and in a lateral tail vein for injection or infusion of test substances. Thirty min before the experiment, the animals receive an intravenous injection of 5 mg/kg furosemide in order to stimulate renin release. During the experiment, the marmosets are sedated with diazepam (0.3 mg/kg i.p.) and kept in restraining boxes. Mean blood pressure is recorded continuously, and heart rate is measured at fixed intervals. The test compound or the standard are injected at various doses by intravenous infusion or administered orally.

Evaluation

Blood pressure is recorded after 30 min of intravenous infusion and 30 min after stopping the infusion. Comparing the changes from pretreatment values after various doses, dose–response curves can be established.

Modifications of the Method

Fischli et al. (1991) monitored arterial pressure in conscious and chronically instrumented monkeys using a telemetry system. One week before the experiment, the animals were anesthetized, and a 3 F high-fidelity pressure-tip transducer (Millar Instruments, Inc.) was inserted into the abdominal aorta through the right femoral artery. Then the catheter was tunneled subcutaneously to the back of the monkey in the interscapular region. The proximal part of the catheter was connected to a transmitter located in a jacket worn by the monkey. The blood pressure was transmitted continuously to a receiver, which transformed the signal to an analogue value of blood pressure.

Linz et al. (1994) reported on the effects of renin inhibitors in anesthetized rhesus monkeys weighing between 5 and 13 kg. The animals are sodium depleted by administration of 10 mg/kg/day furosemide-Na for 6 consecutive days. At day 7, 10 mg/kg furosemide is given i.v. 30 min before the start of the experiment. Anesthesia is induced with 20 mg/kg ketamine hydrochloride i.m. and continued with 40 mg/kg pentobarbitone-Na, slow i.v. drip. After completion of surgical procedures and after insertion of catheters under fluoroscopic control, the following hemodynamic parameters are measured: Pulse rate and systolic and diastolic blood pressures are registered with a transducer (Statham P23 ID) in one femoral artery. A catheter tip manometer (Millar Instruments, Houston, Texas, USA) is introduced into the left ventricular cavity for the determination of left ventricular pressure. Contractility is electronically deduced from left ventricular pressure with appropriate amplifiers (Hellige GmbH, Freiburg, Germany). The electrocardiogram (ECG) from conventional lead II is taken using an ECG transducer (Hellige GmbH). Heart rate is measured from QRS peaks using a biotachometer (Hellige GmbH). Cardiac output is determined using the thermodilution method. Thermodilution is integrated and converted to cardiac output readings by commercially available equipment (HMV7905, Hoyer, Bremen). To determine cardiac output, 2 ml chilled 0–5 °C isotonic glucose solution (5 %) is injected rapidly into the right ventricle by a catheter via the right jugular vein. A thermistor is placed into the aortic arch via the right carotid artery.

Hemodynamics are monitored for 30 min following i.v. injection of various doses of the potential renin inhibitor. At the end of the experiments, the ACE inhibitor ramiprilat 100 µg/kg is given i.v. to probe for an additional blood pressure-lowering effect. Blood samples for the determination of ANG II concentration, renin inhibition, and plasma drug levels are withdrawn at 10, 30, and 60 min after i.v. injection of the renin inhibitor. The volume is replaced by i.v. injections of isotonic glucose solution (5 %). After all data and blood samples have been obtained, animals are sacrificed by an overdose of pentobarbitone-Na.

For experiments after intraduodenal administration, sodium depletion and anesthesia are done as described above. A small side branch of the femoral or radial artery is surgically exposed and cannulated for blood pressure measurements using a pressure transducer (P23 ID). Heart rate is determined from a conventional ECG lead by a biotachometer. Blood samples are withdrawn via a catheter placed into the saphenous vein. A gastric fiberscope (Olympus XP 10) is introduced into the duodenum under visual control, and the renin inhibitor is administered intraduodenally through the service channel of the fiberscope in a volume of 5 ml. Blood samples are withdrawn before and at 15, 30, 45, 60, 90, and 120 min after intraduodenal administration.

Wood et al. (2005) tested an orally effective renin inhibitor (aliskiren) in marmosets. Blood pressure and heart rate were measured by telemetry in conscious animals moving freely in their home cages. Pressure transmitters (AM Unit, model TA11PA-C40, Data Sciences, USA) were implanted into the peritoneal cavity under aseptic conditions and light anesthesia. The sensor catheter was placed in the aorta below the renal artery pointing upstream.

Critical Assessment of the Method

Due to the high species specificity of renin and its substrate, angiotensinogen, renin inhibitors for treatment of hypertension have to be tested in primate models. The marmoset as well as the rhesus monkey have been proven to be suitable models.

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Penile Erection in Rabbits

Purpose and Rationale

The discovery of inhibitors of phosphodiesterase as effective drugs for patients with erectile dysfunction (Klotz et al. 2001; Porst et al. 2001) has stimulated the use of appropriate in vivo animal models. In particular, rabbits have been recommended as models for impotence research (Bischoff and Schneider 2000, 2001; Bischoff 2001; Bischoff et al. 2001; Saenz de Tejada et al. 2001) confirming earlier work in this area (Thielen et al. 1969; Sjöstrand and Klinge 1979; Naganuma et al. 1993; Lin and Lin 1996).

Procedure

Adult male Chinchilla rabbits weighing 3.5–4.5 kg are housed in individual cages for at least 1 week after arrival, at room temperature with water and food ad libitum.

For the study, an indwelling catheter filled with saline is inserted into a marginal ear vein and taped in position. The drugs are injected into the ear vein, followed by a small volume of saline. The time is noted and at appropriate times the animal gently removed from the cage and held by one research worker. The rabbit penis is not visible when it is not erect (Naganuma et al. 1993). However, when erection occurs, it is possible to examine the pudendal area and measure the length of the uncovered penile mucosa with sliding calipers.

Evaluation

Penile erection is evaluated by measuring the length to the nearest millimeter of the uncovered penile mucosa with a sliding caliper at 5, 10, 15, 30, 50, 60, 90, and 120 min after administration of the test compounds and continued hourly for up to 5 h. Mean values are calculated and results expressed as means ± SEM. The area under the curve is calculated by an integration program.

Modifications of the Method

Choi et al. (2002) compared the efficacy of vardenafil and sildenafil in facilitating penile erection in **anesthetized rabbits**. Penile erections were elicited by submaximal pelvic nerve stimulation every 5 min for 30 min. Response was assessed by continuously recording intracavernosal pressure and systemic arterial pressure.

Min et al. (2000) tested the augmentation of pelvic nerve-mediated sexual arousal in anesthetized **female rabbits** by sildenafil. The following parameters were measured before, during, and after pelvic nerve stimulation at 4, 16, and 32 Hz: (1) hemoglobin concentration and oxygen saturation in female genital (vaginal, labial, clitoral) tissues by laser oximetry, (2) clitoral blood flow by laser Doppler flowmetry, (3) vaginal luminal pressure by a balloon catheter pressure transducer, and (4) vaginal lubrication by tampon.

Carter et al. (1998) tested the effect of the selective phosphodiesterase type 5 inhibitor sildenafil on erectile dysfunction in **pentobarbital-anesthetized dogs**. Increases in intracavernosal pressure in the corpus cavernosum and penile blood flow were induced by pelvic nerve stimulation over a frequency range of 1–16 Hz. The effects of increasing doses of sildenafil on electric-stimulated intracavernosal pressure, penile blood flow, blood pressure, and heart rate were evaluated.

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