Patch-Clamp and Voltage-Clamp Techniques

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

Contents

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

F.J. Hock (ed.), *Drug Discovery and Evaluation: Pharmacological Assays*,
DOI 10.1007/978-3-319-05392-9_146

Patch-Clamp Technique

Purpose and Rationale

The introduction of the patch-clamp technique (Neher and Sakmann 1976) revolutionized the study of cellular physiology by providing a high-resolution method of observing the function of individual ionic channels in a variety of normal and pathological cell types. By the use of variations of the basic recording methodology, cellular function and regulation can be studied at a molecular level by observing currents through individual ionic channels (Liem et al. 1995; Sakmann and Neher 1995).

The most intriguing method is called the "on-cell" or "cell-attached" configuration, because ion channels can be recorded on an intact cell (Jackson 1993). This mode is well suited for investigation of ion channels that are activated by hormonal stimulation and triggered by intracellular second messengers.

Another versatile mode is the "cell-excised" configuration (Hamill 1993). It is obtained by suddenly removing the patch pipette from the cell, so that the membrane patch is pulled off the cell. This mode easily allows the investigator to expose the channel proteins to drugs by changing the bath solution. The single-channel currents are recorded on a videotape and are analyzed off-line by a computer system. Various parameters are evaluated, such as the single-channel conductance, the open and closed times of the channel, and the open-state probability, which is the percentage of time the channel stays in its open state.

In addition to these modes, which enable the recording of single-channel currents, it is also possible to measure the current flowing through the entire cell. This "whole-cell mode" is obtained by rupturing the membrane patch in the cell-attached mode (Hamill et al. 1981; Dietzel et al. 1993). This is achieved by applying suction to the interior of the patch pipette. The "whole-cell mode" allows not only the recording of electrical current but also the measurement of cell potential. Moreover, the cell interior is dialyzed by the electrolyte solution contained in the patch pipette.

The fabrication of patch-clamp pipettes has been described by Sakmann and Neher (1995) and Cavalié et al. (1993).

Variations of the patch-clamp technique have been used to study neurotransmitter transduction mechanisms (Smith 1995).

High-throughput methods are required when developing drugs that work on ion-channel function (Mathes 2003; Bennett and Guthrie 2003). Patch clamping suffers from low throughput, which is not acceptable for drug screening.

Fertig et al. (2002) and Brueggemann et al. (2004, 2006) presented nanopatch-clamp technology, which is based on a planar, microstructured glass chip, which enables automatic whole-cell patch-clamp experiments. Planar glass substrates containing a single microaperture produced by ion track etching are used to record currents through ion channels in living mammalian cells.

Falconer et al. (2002) reported high-throughput screening for ion-channel modulators setting up a Beckman/Sagian core system to fully automate functional fluorescence-based assays that measure ion-channel function. Voltage-sensitive fluorescent probes were applied and the activity of channels was measured using Aurora's Voltage/Ion Probe Reader (VIPR). The system provides a platform for fully automated high-throughput screening as well as pharmacological characterization of ion-channel modulators.

Schroeder et al. (2003) described a high-throughput electrophysiology measurement platform consisting of computer-controlled fluid handling, recording electronics, and processing tools capable of whole-cell voltage-clamp recordings from thousands of individual cells per day. The system uses a planar, multiwell substrate (a PatchPlate). The system positions one cell into a hole separating two fluid compartments in each well of the substrate. Voltage control and current recordings from the cell membrane are made subsequent to gaining access to the cell interior by applying a permeabilizing agent to the intracellular side.

Willumsen's group recommended ion-channel screening with QPatch (Asmild et al. 2003; Kutchinsky et al. 2003; Krzywkowski et al. 2004). This system claims to allow fast and

accurate electrophysiological characterization of ion channels, e.g., for determination of IC_{50} values for ion-channel blockers. The system comprises 16 parallel patch-clamp sites, each based on a silicon chip with a micro-etched patch-clamp hole. Intra- and extracellular fluids are administered by laminar flow through integrated miniature flow channels.

Spencer et al. (2012) described a novel microfluidic automated patch-clamp device; the IonFlux™ system utilizes microfluidic channels molded into a polymeric substrate that eliminates the necessity of internal robotic liquid handling.

References and Further Reading

Asmild M, Oswald N, Krzywkowski FM, Friis S, Jacobsen RB, Reuter D, Taboryski R, Kutchinsky J, Vestergaard RK, Schroder RL, Sorensen CB, Bech M, Korsgaard MP, Willumsen NJ (2003) Upscaling and automation of electrophysiology: toward high throughput screening in ion channel drug discovery. Receptor Channels 9:49–58

Bennett PB, Guthrie HRE (2003) Trends in ion channel drug discovery: advances in screening technologies. Trends Biotechnol 21:563–569

Brueggemann A, George M, Klau M, Beckler M, Steimdl J, Behrends JC, Fertig N (2004) Ion channel drug discovery and research. The automated nano-patch-clamp technology. Curr Drug Discov Technol 1:91–96

Brüggemann A, Stoelzle S, George M, Behrends JC, Fertig N (2006) Microchip technology for automated and parallel patch-clamp recording. Small 2:840–846

Cavalié A, Grantyn R, Lux HD (1993) Fabrication of patch clamp pipettes. In: Kettenmann H, Grantyn R (eds) Practical electrophysiological methods. Wiley, New York, pp 235–240

Dietzel ID, Bruns D, Polder HR, Lux HD (1993) Voltage clamp recording. In: Kettenmann H, Grantyn R (eds) Practical electrophysiological methods. Wiley, New York, pp 256–262

Falconer M, Smith F, Surah-Narwal S, Congrave G, Liu Z, Hayter P, Ciaramella G, Keighley W, Haddock P, Waldron G, Sewing A (2002) High-throughput screening for ion channel modulators. J Biomol Screen 7:460–465

Fertig N, Klau M, George M, Blick RH, Behrends JC (2002) Activity of single ion channel proteins detected with a planar microstructure. Appl Phys Lett 81:4865–4867

Hamill OP (1993) Cell-free patch clamp. In: Kettenmann H, Grantyn R (eds) Practical electrophysiological methods. Wiley, New York, pp 284–288

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85–100

Jackson MB (1993) Cell-attached patch. In: Kettenmann H, Grantyn R (eds) Practical electrophysiological methods. Wiley, New York, pp 279–283

Krzywkowski K, Schroder RL, Ljungstrom T, Kutchinsky J, Friis S, Vestergaard RK, Jacobsen RB, Pedersen S, Helix N, Sorensen CB, Bech M, Willumsen NJ (2004) Automation of the patch-clamp technique: technical validation through identification and characterization of potassium channel blockers. Biophys J 86:483a

Kutchinsky J, Friis S, Asnild M, Taboryski R, Pedersen S, Vestergaard RK, Jacobsen RB, Krzywkowski K, Schroder RL, Ljungstrom T, Helix N, Sorensen CB, Bech M, Willumsen NJ (2003) Characterization of potassium channel modulators with QPatch automated patch-clamp technology system characteristics and performance. Assay Drug Dev Technol 1:685–693

Liem LK, Simard JM, Song Y, Tewari K (1995) The patch clamp technique. Neurosurgery 36:382–392

Mathes C (2003) Ion channels in drug discovery and development. Drug Discov Today 8:1022–1024

Neher E, Sakmann B (1976) Single-channel currents recorded from membranes of denervated frog muscle fibres. Nature 260:799–802

Sakmann B, Neher E (1995) Single-cell recording. Plenum, New York

Schroeder K, Neagle B, Trezise DJ, Worley J (2003) Ion-Works™ HT: a new high-throughput electrophysiology measurement platform. J Biomol Screen 8:50–64

Smith PA (1995) Methods for studying neurotransmitter transduction mechanisms. J Pharmacol Toxicol Methods 33:63–73

Spencer CI, Li N, Chen Q, Johnson J, Nevill T, Kammonen J, Ionescu-Zanetti C (2012) Ion channel pharmacology under flow: automation via well-plate microfluidics. Assay Drug Dev Technol 10(4):313–324

Patch-Clamp Technique in Isolated Cardiac Myocytes

Purpose and Rationale

The generation of an action potential in heart muscle cells depends on the opening and closing of ion-selective channels in the plasma membrane. The patch-clamp technique enables the investigation of drug interactions with ion-channel-forming proteins at the molecular level.

Procedure

Isolated cells from ventricular muscle of rat and guinea pig are prepared as described by Yazawa et al. (1990). Animals are sacrificed by cervical dislocation. Hearts are dissected and mounted on a Langendorff-type apparatus and perfused first with Tyrode solution (in mM: 143 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.25 NaH_2PO_4, 5 HEPES, pH adjusted to 7.4 with NaOH) at 37 °C for 3 min at a hydrostatic pressure of 60–70 cmH_2O, then with nominally Ca^{2+}-free Tyrode solution (no Ca^{2+} is added) for 5–7 min, and finally, with nominally Ca^{2+}-free Tyrode solution containing 0.12–0.2 mg/ml collagenase (Sigma, type I). After 15–20 min of collagenase treatment, the heart is now soft and is washed with storage solution (in mM: 70 KOH, 50 L-glutamic acid, 40 KCl, 20 taurine, 20 KH_2PO_4, 3 $MgCl_2$, 10 glucose, 10 HEPES, 0.5 EGTA, pH adjusted to 7.4 with KOH). The ventricles are cut into pieces (about 5 mm × 5 mm) and poured into a beaker. The myocytes are dispersed by gently shaking the beaker and filtration through a nylon mesh (365 μm). Then, the myocytes are washed twice by centrifugation at 600–1,000 rpm (about 90 *g*) for 5 min and kept at room temperature. The rod shape of the cell and the clear striations of sarcomeres are important criteria for selecting viable cells for the assay. Experiments are performed at 35 °C–37 °C.

For investigation with the patch-clamp technique (Neher and Sakmann 1976; Hamill et al. 1981), the isolated cells are placed into a thermostat-controlled chamber, mounted on the stage of an inverted microscope equipped with differential interference contrast optics. Under optical control (magnification 400×), a glass micropipette, having a tip opening of about 1 μm, is placed onto the cell. The patch pipettes are fabricated from borosilicate glass tubes (outer diameter 1.5 mm, inner diameter 0.9 mm) by means of an electrically heated puller. In order to prevent damage of the cell membrane, the tip of the micropipette is fire polished, by moving a heated platinum wire close to the tip. The patch pipette is filled with either high-NaCl or KCl solution and is mounted on a micromanipulator. A silver chloride wire connects the pipette solution to the head stage of an electronic amplifier. A second silver chloride wire is inserted into the bath and serves a ground electrode.

After establishing contact with the cell membrane, a slight negative pressure is applied to the inside of the patch pipette by means of a syringe. Consequently, a small patch of membrane is slightly pulled into the opening of the micropipette, and close contact between the glass and membrane is formed, leading to an increase of the electrical input resistance into the giga-ohm range (about 10^{10} Ω). This high input resistance enables the recording of small electrical currents in the range of picosiemens (10^{-12} S), which flow through channel-forming proteins situated in the membrane patch. The electrical current is driven by applying an electrical potential across the membrane patch and/or by establishing an appropriated chemical gradient for the respective ion species.

The patch-clamp method allows one to investigate the interaction of drugs with all ion channels involved in the functioning of the heart muscle cell (K^+, Na^+, Ca^{2+}, and eventually Cl^- channels). Moreover, the different types of K^+ channels existing in cardiomyocytes can be distinguished by their different single-channel characteristics or by appropriate voltage-pulse protocols in the whole-cell mode.

Evaluation

Concentration–response curves of drugs which inhibit or activate ion channels can be recorded either at the single-channel level or by measuring the whole-cell current. IC_{50} and EC_{50} values (50 % inhibition or activation, respectively) can be obtained with both methods.

Modifications of the Method

The patch-clamp technique has been used for evaluation of antiarrhythmic agents (Bennett et al. 1987; Anno and Hondeghem 1990; Gwilt et al. 1991).

Gögelein et al. (1998) used isolated ventricular myocytes from guinea pigs to study a cardioselective inhibitor of the ATP-sensitive potassium channel.

Multiple types of calcium channels have been identified by patch-clamp experiments (Tsien et al. 1988).

The effects of potassium channel openers have been measured (Terzic et al. 1994).

Ryttsén et al. (2000) characterized electroporation of single NG108–15 cells with carbon-fiber microelectrodes by patch-clamp recordings and fluorescence microscopy.

Monyer and Lambolez (1995) reviewed the molecular biology and physiology at the single-cell level, discussing the value of the polymerase chain reaction at the single-cell level and the use of patch pipettes for collecting the contents of a single cell on which the reverse transcription is performed.

The patch-clamp technique was found to be very versatile in the investigation of ion channels in atrial myocytes, especially from dogs or humans. Cells were obtained from atria either in sinus rhythm or in atrial fibrillation (reviewed in Bosch et al. 1999).

References and Further Reading

Anno T, Hondeghem LM (1990) Interaction of flecainide with guinea pig cardiac sodium channels. Circ Res 66:789–803

Bennett PB, Stroobandt R, Kesteloot H, Hondeghem LM (1987) Sodium channel block by a potent, new antiarrhythmic agent, transcainide, in guinea pig ventricular myocytes. J Cardiovasc Pharmacol 9:661–667

Bosch RF, Zeng X, Grammer JB, Popovic K, Mewis C, Kühlkamp V (1999) Ionic mechanisms of electrical remodeling in human atrial fibrillation. Cardiovasc Res 44:121–131

Gögelein H, Hartung J, Englert HC, Schölkens BA (1998) HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. Part I. Effects on cardiomyocytes, coronary flow and pancreatic β-cells. J Pharmacol Exp Ther 286:1453–1464

Gwilt M, Dalrymple HW, Burges RA, Blackburn KJ, Dickinson RP, Cross PE, Higgins AJ (1991) Electrophysiologic properties of UK-66,914, a novel class III antiarrhythmic agent. J Cardiovasc Pharmacol 17:376–385

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85–100

Monyer H, Lambolez B (1995) Molecular biology and physiology at the single-cell level. Curr Opin Neurobiol 5:382–387

Neher E, Sakmann B (1976) Single-channel currents recorded from membranes of denervated frog muscle fibres. Nature 260:799–802

Pallotta BS (1987) Patch-clamp studies of ion channels. In: Meltzer HY (ed) Psychopharmacology: the third generation of progress. Raven, New York, pp 325–331

Ryttsén F, Farre C, Brennan C, Weber SG, Nolkrantz K, Jardemark K, Chiu DT, Orwar O (2000) Characterization of single-cell

electroporation by using patch-clamp and fluorescence microscopy. Biophys J 79:1993–2001
Terzic A, Jahangir A, Kurachi Y (1994) HOE-234, a second generation K^+ channel opener, antagonizes the ATP-dependent gating of cardiac ATP-sensitive K^+ channels. J Pharmacol Exp Ther 268:818–825
Tsien RW, Lipscombe D, Madison DV, Bley RK, Fox AP (1988) Multiple types of neuronal calcium channels and their selective modulation. Trends Neurosci 11:431–438
Yazawa K, Kaibara M, Ohara M, Kameyama M (1990) An improved method for isolating cardiac myocytes useful for patch-clamp studies. Jpn J Physiol 40:157–163

Voltage-Clamp Studies on Sodium Channels

Purpose and Rationale

The epithelial Na^+ channel plays an important role in epithelial Na^+ absorption in the distal colon, urinary bladder, salivary and sweat ducts, respiratory tract, and, most importantly, distal tubules of the kidney (Catterall 1986; Palmer 1992). Regulation of this epithelial Na^+ channel has a major impact on Na^+ balance, blood volume, and blood pressure. Inhibition of epithelial Na^+ channel expression is used for the treatment of hypertension (Endou and Hosoyamada 1995). Busch et al. (1995) studied the blockade of epithelial Na^+ channels by triamterenes using two-microelectrode voltage-clamp experiments in *Xenopus* oocytes expressing the three homologous subunits (α, β, and γ) of the rat epithelial Na^+ channel (rENaC).

Procedure

Xenopus laevis oocytes are injected with the appropriate cRNA encoding for the α-, β-, and γ-subunits Canessa et al. (1994) of the rat epithelial Na^+ channel (rENaC). The cRNA for the wild-type α-subunit and its deletion mutant Δ278–273 is always coinjected with an equal amount of β- and γ-subunit cRNA (10 ng/oocyte).

Then, 2–8 days after cRNA injection, the two-microelectrode voltage-clamp method is used to record currents from *Xenopus* oocytes. Recordings are performed at 22 °C using a Geneclamp amplifier (Axon Instruments, Foster City, CA, USA) and MacLab D/A converter and software for data acquisition and analysis (ADInstruments, Castle Hill, Australia). The ND 96 solution (control) contains (in mM) NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, and HEPES 5, pH 7.0. In some experiments, Na^+ is replaced by *N*-methyl-D-glucamine (NMDG) solution. The microelectrodes are filled with 3 M KCl solution and have resistances in the range 0.5–0.9 MΩ. Chemicals (e.g., triamterene as standard) are added at concentrations between 0.2 and 100 µM. The amplitude of the induced currents varies considerably, depending on the day of channel expression and the batch of oocytes. The mutant channel induces considerably smaller currents than the wild-type channel. The total Na^+ current amplitude is determined at least once for each experimental day by superfusion with NMDG solution or with 3 µM or 5 µM amiloride solution at the beginning and at the end of each set of experiments.

Evaluation

Data are presented as means ±SEM. A paired Student's *t*-test is used. The level of statistical significance is set at $P < 0.05$.

Modifications of the Method

Nawada et al. (1995) studied the effects of a sodium, calcium, and potassium antagonistic agent on the sodium current by the whole-cell voltage-clamp technique (tip resistance = 5 MΩ $[Na]_i$ and $[Na]_o$ 10 mmol/l at 20 °C) in isolated guinea pig ventricular cells.

Sunami and Hiraoka (1996) studied the mechanism of cardiac Na^+ channel block by a charged class I antiarrhythmic agent, in guinea pig ventricular myocytes using patch-clamp techniques in the whole-cell, cell-attached, and inside-out configurations.

Erdõ et al. (1996) compared the effects of *Vinca* derivatives on voltage-gated Na^+ channels in cultured cells from rat embryonic cerebral cortex. Effects on Na^+ currents were measured by applying voltage steps (20 ms duration) to −10 mV from a holding potential of −70 mV every 20 s. Steady-state inactivation curves were obtained by clamping the membrane at one of a series of 15-s prepulse potentials, followed 1 ms later by a 20-ms test pulse to −10 mV.

Ragsdal et al. (1993) examined the actions of a Na^+ channel blocker in whole-cell voltage-clamp recordings from Chinese hamster ovary cells transfected with a cDNA encoding the rat brain type IIA Na^+ channel and from dissociated rat brain neurons.

Taglialatela et al. (1996) studied cloned voltage-dependent Na^+ currents expressed in *Xenopus* oocytes upon injection of the cRNA encoding α-subunits from human and rat brain.

Wang et al. (1997) investigated pharmacological targeting of long QT mutant sodium channels.

Eller et al. (2000) measured the effects of a calcium antagonist on inward Na^+ currents (I_{Na}) in GH3 cells with the whole-cell configuration of the patch-clamp technique. I_{Na} was recorded after depolarization from a holding potential of −80 mV to a test potential of +5 mV. Initial "tonic" block (resting state-dependent block) was defined as peak I_{Na} inhibition during the first pulse 2 min after drug application as compared with I_{Na} in the absence of drug. "Use (frequency)-dependent" block of I_{Na} was measured during trains of 5- or 50-ms test pulses (3 Hz) applied from −80 mV to a test potential of +5 mV after a 2-min equilibrium period in the drug-containing solution. Use-dependent block was expressed as the percentage decrease of peak I_{Na} during the last pulse of the train as compared with I_{Na} during the first pulse.

Khalifa et al. (1999) characterized the effects of an antidepressant agent on the fast inward current (I_{Na}) in isolated guinea pig ventricular myocytes. Currents were recorded in the whole-cell configuration of the patch-clamp technique in the presence of Ca^{2+} and K^+ channel blockers.

Haeseler et al. (1999) measured the effects of 4-chloro-*m*-cresol, a preservative added to a wide variety of drugs, on heterologously expressed wild-type paramyotonia congenita (R1448H) and hyperkalemic periodic paralysis (M1360V) mutant α-subunits of human muscle sodium channels using whole-cell and inside-out voltage-clamp experiments.

Song et al. (2000) studied the effects of *N*-ethylmaleimide, an alkylating agent to protein sulfhydryl groups, on tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels in rat dorsal root neurons using the whole-cell configuration of the patch-clamp technique. Rats at the age of 2–6 days were anesthetized with isoflurane, and the spinal cord was removed and cut longitudinally. Dorsal root ganglia were plucked from the area between the vertebrae of the spinal column and incubated in phosphate-buffered saline solution containing 2.5 mg/ml trypsin at 37 °C for 30 min. After enzyme treatment, ganglia were rinsed with Dulbecco's Modified Eagle Medium supplemented with 10 % horse serum. Single cells were mechanically dissociated by trituration with a fire-polished Pasteur pipette and plated on poly-L-lysine-coated glass coverslips. Cells attached to the coverslips were transferred into a recording chamber on the stage of an inverted microscope. Ionic currents were recorded under voltage-clamp conditions by the whole-cell patch-clamp technique. The solution in the pipette contained (in mM) CsCl 125, NaF 20, HEPES 5, and EGTA 5. The pH was adjusted to 7.2 with CsOH and the osmolarity was 279 mosmol/l on average. The external solution contained (in mM) NaCl 50, choline chloride 90, tetramethylammonium chloride 20, D-glucose 5, HEPES 5, $MgCl_2$ 1, and $CaCl_2$ 1. Lanthanum ($LaCl_3$, 10 μM) was used to block calcium channel current. The solution was adjusted to pH 7.4 with tetramethylammonium hydroxide and the osmolarity was 304 mosmol/l on average. An Ag–AgCl pellet/3 M KCl-agar bridge was used for the reference electrode. Membrane currents were recorded using an Axopatch-1D amplifier. Signals were digitized by a 12-bit analogue-to-digital interface, filtered with a low-pass Bessel filter at 5 kHz, and sampled at 50 kHz using pCLAMP6 software (Axon Instruments) on an IBM-compatible PC. Series resistance was

compensated 60–70 %. Capacitive and leakage currents were subtracted by using a P + P/4 procedure (Bezanilla and Armstrong 1977). The liquid junction potential between internal and external solutions was on average −1.7 mV. TTX (100 nM) was used to separate TTX-R sodium currents from TTX-S sodium currents. For the study of TTX-S sodium channels, cells that expressed only TTX-S sodium channels were used. TTX-S sodium channels were completely inactivated within 2 ms when currents were evoked by depolarizing steps to 0 mV, while TTX-R sodium channels persisted for more than 20 ms. The difference in kinetics was used to identify the type of sodium current.

Abriel et al. (2000) described the molecular pharmacology of the sodium channel mutation DI790G linked to long QT syndrome.

Makielski et al. (2003) showed that a ubiquitous splice variant and a common polymorphism affect heterologous expression of recombinant human SCN5AS heart sodium channels.

Viswanathan et al. (2001) studied gating mechanisms for flecainide action in *SNCN5A*-linked arrhythmia syndromes.

References and Further Reading

Abriel H, Wehrens XHT, Benhorin J, Kerem B, Kass RS (2000) Molecular pharmacology of the sodium channel mutation DI790G linked to the long-QT- syndrome. Circulation 102:921–925

Bezanilla F, Armstrong CM (1977) Inactivation of the sodium channel: I. Sodium current experiments. J Gen Physiol 70:549–566

Busch AE, Suessbrich H, Kunzelmann K, Hipper A, Greger R, Waldegger S, Mutschler E, Lindemann B, Lang F (1995) Blockade of epithelial Na^+ channels by triamterenes – underlying mechanisms and molecular basis. Pflugers Arch 432:760–766

Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC (1994) Amiloride-sensitive N^+ channel is made of three homologous subunits. Nature 367:463–467

Catterall WA (1986) Molecular properties of voltage-sensitive sodium channels. Annu Rev Biochem 55:953–985

Eller P, Berjukov S, Wanner S, Huber I, Hering S, Knaus HG, Toth G, Kimball SD, Striessnig J (2000) High affinity interaction of mibefradil with voltage-gated calcium and sodium channels. Br J Pharmacol 130:669–677

Endou H, Hosoyamada M (1995) Potassium-retaining diuretics: aldosterone antagonists. In: Greger RH, Knauf H, Mutschler E (eds) Handbook of experimental pharmacology, vol 117. Springer, Berlin/Heidelberg/New York, pp 335–362

Erdõ SL, Molnár P, Lakics V, Bence JZ, Tömösközi Z (1996) Vincamine and vincanol are potent blockers of voltage-gated Na^+ channels. Eur J Pharmacol 314:69–73

Haeseler G, Leuwer M, Kavan J, Würz A, Dengler R, Piepenbrock S (1999) Voltage-dependent block of normal and mutant muscle sodium channels by 4-Chloro-m-Cresol. Br J Pharmacol 128:1259–1267

Khalifa M, Daleau P, Turgeon J (1999) Mechanism of sodium channel block by venlafaxine in guinea pig ventricular myocytes. J Pharmacol Exp Ther 291:280–284

Makielski JC, Ye B, Valdivia CR, Pagel MD, Pu J, Tester DJ, Ackerman MJ (2003) A ubiquitous splice variant and a common polymorphisms affect heterologous expression of recombinant human SCN5AS heart sodium channels. Circ Res 93:821–828

Nawada T, Tanaka Y, Hisatome I, Sasaki N, Ohtahara A, Kotake H, Mashiba H, Sato R (1995) Mechanism of inhibition of the sodium current by bepridil in guinea-pig isolated ventricular cells. Br J Pharmacol 116:1775–1780

Palmer LG (1992) Epithelial Na channels: function and diversity. Annu Rev Physiol 54:51–66

Ragsdal DS, Numann R, Catterall WA, Scheuer T (1993) Inhibition of Na^+ channels by the novel blocker PD85,639. Mol Pharmacol 43:949–954

Song J-H, Jang Y-Y, Shin Y-K, Lee C-S, Chung S (2000) N-Ethyl-maleimide modulation of tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels in rat dorsal root neurons. Brain Res 855:267–273

Sunami A, Hiraoka M (1996) Blockade of cardiac Na^+ channels by a charged class I antiarrhythmic agent, bisaramil: possible interaction of the drug with a pre-open closed state. Eur J Pharmacol 312:245–255

Taglialatela M, Ongini E, Brown AM, di Renzo G, Annunziato L (1996) Felbamate inhibits cloned voltage-dependent Na^+ channels from human and rat brain. Eur J Pharmacol 316:373–377

Viswanathan PV, Bezzina CR, George AL Jr, Roden DM, Wilde AAM, Balser JR (2001) Gating mechanisms for flecainide action in SNCN5A-linked arrhythmia syndromes. Circulation 104:1200–1205

Wang DW, Yazawa K, Makita N, George AL Jr, Bennett PB (1997) Pharmacological targeting of long QT mutant sodium channels. J Clin Invest 99:1714–1720

Voltage-Clamp Studies on Potassium Channels

Purpose and Rationale

Potassium channels represent a very large and diverse collection of membrane proteins which participate in important cellular functions regulating neuronal and cardiac electrical patterns, release of neurotransmitters, muscle contractility, hormone secretion, secretion of fluids, and modulation of signal transduction pathways. The main categories of potassium channels are gated by voltage or an increase of intracellular calcium concentration (Escande and Henry 1993; Kaczorowski and Garcia 1999; Alexander et al. 2001). For ATP-sensitive potassium channels, see section "Interaction with β-Cell Plasma Membranes and K_{ATP} Channels," chapter "▶ Assays for Insulin and Insulin-Like Metabolic Activity Based on Hepatocytes, Myocytes and Diaphragms".

The delayed outward potassium current in heart muscle cells of several species is made up of a **rapidly** (I_{Kr}) and a **slowly** (I_{Ks}) activating component (Sanguinetti and Jurkiewicz 1990; Wang et al. 1994; Gintant 1996; Lei and Brown 1996; Carmeliet and Mubagawa 1998). Several potent and selective blockers of the I_{Kr} channel have been shown to prolong the effective refractory period but have a reverse rate-dependent activity with both normal and elevated extracellular potassium concentrations (Colatsky et al. 1990). Inhibitors of the slow component I_{Ks} were developed in order to circumvent the negative rate dependence of I_{Kr} channel blockers in the effective refractory period (Busch et al. 1996; Suessbrich et al. 1996, 1997; Bosch et al. 1998). Gögelein et al. (2000) studied the effects of a potent inhibitor of I_{Ks} channels in *Xenopus* oocytes and guinea pig ventricular myocytes.

Procedure

Studies in *Xenopus* oocytes are performed with the two-microelectrode voltage-clamp method. For isolation of the oocytes, the toads are anesthetized using a 1 g/l solution of 3-aminobenzoic acid ethyl ester and placed on ice. A small incision is made to retrieve sacs of oocytes and is subsequently closed with absorbable surgical suture. On waking up, the toads are placed back into the aquarium. The ovaries are cut up into small pieces, and the oocytes are washed in Ca^{2+}-free Or-2 solution (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES; pH 7.4) and subsequently digested in Or-2 containing collagenase A (1 mg/ml, Worthington, type II) until follicles are not longer detectable on the oocyte's surface. The oocytes are stored at 18 °C in recording solution ND-96 (NaCl 96 mM, KCl 2 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1 mM, HEPES 5 mM, pH 7.4) with added sodium pyruvate (275 mg/l), theophylline (90 mg/l), and gentamicin (50 mg/l).

For electrophysiological recordings, the two-microelectrode voltage-clamp configuration is used to record ion currents from *Xenopus* oocytes. Injection of cRNA is performed according to Methfessel et al. (1986) and Golding (1992). Oocytes are injected individually with cRNA encoding for the human protein minK, guinea pig Kir2.1, human H*erg*, human Kv1.5, mouse Kv1.3, or human HNC2. In the case of minK, the functional potassium channel is a heteromultimer composed of the endogenous

(*Xenopus*) KvLQT1 and the injected human minK. This heteromultimeric potassium current is then called I_{Ks} (Barhanin et al. 1996; Sanguinetti et al. 1996).

The electrophysiological recordings are performed at room temperature, using a Geneclamp amplifier (Axon Instruments) and MacLab D/A converter. The amplitudes of the recorded currents are measured at the end of the test voltage steps. To amplify the inward potassium current through Kir2.1 and HNC2, the external potassium concentration is raised to 10 mM KCl and the NaCl concentration lowered to 88 mM (ND-88). The microelectrodes are filled with 3 M KCl and have a resistance between 0.5 MΩ and 1 MΩ. During the recordings the oocytes are continuously perfused with ND-96 (or ND-88 in the case of Kir2.1 and HCN2). The test compounds are dissolved in dimethyl sulfoxide (DMSO) and added to the buffer ND-96 or ND-88. The current amplitude is determined after 5 min of wash-in time.

For the isolation of *ventricular myocytes*, guinea pigs (weight about 400 g) or Sprague–Dawley rats of either sex are sacrificed by cervical dislocation. The hearts are dissected and perfused retrogradely via the aorta at 37 °C: first, with nominally Ca^{2+}-free Tyrode solution (in mmol/l: 143 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 0.25 NaH_2PO_4, 10 glucose, 5 HEPES, pH 7.2) and then with Tyrode solution containing 20 mmol/l Ca^{2+} and 3 mg/ml collagenase type CLS II (Biochrom, Berlin, Germany). After 5–10 min collagenase treatment, the ventricles are cut up into small pieces in the storage solution (in mmol/l: 50 L-glutamic acid monopotassium salt, 40 KCl, 20 taurine, 20 KH_2PO_4, 1 $MgCl_2$, 10 glucose, 0.2 EGTA, pH 7.2). The myocytes are then dispersed by gentle shaking followed by filtration through a nylon mesh (365 μm). The cells are finally washed twice by centrifugation at 90 *g* for 5 min and kept in the storage solution at room temperature.

Whole-cell currents are recorded in the tight-seal whole-cell mode of the patch-clamp technique, using an EPC-9 amplifier (HEKA Elektronik, Lambrecht, Germany). Patch pipettes are pulled from borosilicate glass capillaries (wall thickness 0.3 mm, outer diameter 1.5 mm) and their tips are fire polished. Series resistance is in the range of 1–10 MΩ and 50 % compensated by means of the EPC's compensation circuit.

The I_{Ks}, I_{Kr}, and I_{K1} currents in guinea pig ventricular myocytes are investigated. The voltage pulses for recording the current components are as follows: I_{Ks} current, holding potential −80 mV to −50 mV (200 ms) to +60 mV (3 s) to −40 mV (2 s) to −80 mV; I_{Kr} current, holding potential −80 mV to −50 mV (200 ms) to −10 mV (3 s) to −40 mV (2 s) to −80 mV (I_{Kr} is evaluated as the tail current evoked by a voltage pulse from −10 mV to −40 mV); and I_{K1} current, holding potential −80 mV to −120 mV (200 ms) to −80 mV. In order to suppress the L-type Ca^{2+} current, 5 mmol/l nifedipine is added to the bath solution.

Evaluation

All average data are presented as means ± SEM. Student's *t*-test is used to determine the significance of paired observations. Differences are considered as significant at $P < 005$.

Modifications of the Method

Using the whole-cell configuration of the patch-clamp technique, Grissmer et al. (1994) analyzed the biophysical and pharmacological properties of five cloned voltage-gated K^+ channels stably expressed in mammalian cell lines.

Sanchez-Chapula (1999) studied the block of the transient outward K^+ channel (I_{to}) by disopyramide in isolated rat ventricular myocytes using whole-cell patch-clamp techniques.

Using the patch-clamp technique, Cao et al. (2001) investigated the effects of a centrally acting muscle relaxant and structurally related compounds on recombinant small-conductance Ca^{2+}-activated K^+ channels (rSK2 channels) in HEK mammalian cells.

Tagliatela et al. (2000) discussed the block of the K^+ channels encoded by the human *ether-á-go-go-related* gene (HERG), termed $K_{V(r)}$, which are the

molecular determinants of the rapid component of the cardiac repolarizing current $I_{K(Vr)}$, involved in the cardiotoxic potential and CNS effects of first-generation antihistamines and may be therapeutic targets for antiarrhythmic agents (Vandenberg et al. 2001; Zhou et al. 2005).

Chabbert et al. (2001) investigated the nature and electrophysiological properties of Ca^{2+}-independent depolarization-activated potassium currents in acutely isolated mouse vestibular neurons using the whole-cell configuration of the patch-clamp technique. Three types of currents were identified.

Furthermore, Longobardo et al. (1998) studied the effects of a quaternary bupivacaine derivative on delayed rectifier K^+ currents stably expressed in Ltk^- cells using the whole-cell configuration of the patch-clamp technique.

Moreno et al. (2003) studied the effects of a selective angiotensin II type 1 receptor antagonist on cloned potassium channels involved in human cardiac repolarization.

Sanchez-Chapula et al. (2002) investigated the voltage-dependent block of wild-type and mutant HERG K^+ channels by the antimalarial compound chloroquine.

Anson et al. (2004) published molecular and functional characterization of common polymorphism in HERG (KCNH2) potassium channels.

For more information on the evaluation of HERG potassium channels in safety pharmacology, Champeroux et al. (2013).

References and Further Reading

Alexander S, Peters J, Mathie A, MacKenzie G, Smith A (2001) TiPS nomenclature supplement 2001

Anson BD, Ackerman MJ, Tester DJ, Will ML, Delisle BP, Anderson CL, January CT (2004) Molecular and functional characterization of common polymorphism in HERG (KCNH2) potassium channels. Am J Physiol 286: H2434–H2441

Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G (1996) KvLQT1 and IsK (minK) proteins associate to form the IKs cardiac potassium current. Nature 384:78–80

Bosch RF, Gaspo R, Busch AE, Lang HJ, Li R-G, Nattel S (1998) Effects of the chromanol 293B, a selective blocker of the slow component of the delayed rectifier K^+ current, on repolarization in human and guinea pig ventricular myocytes. Cardiovasc Res 38:441–450

Busch AE, Suessbrich H, Waldegger S, Sailer E, Greger R, Lang HJ, Lang F, Gibson KJ, Maylie JG (1996) Inhibition of I_{Ks} in guinea pig cardiac myocytes and guinea pig I_{sK} channels by the chromanol 293B. Pflügers Arch 432:1094–1096

Cao Y-J, Dreixler JC, Roizen JD, Roberts MT, Houamed KM (2001) Modulation of recombinant small-conductance Ca^{2+} -activated K^+ channels by the muscle relaxant chlorzoxazone and structurally related compounds. J Pharmacol Exp Ther 296:683–689

Carmeliet A, Mubagawa K (1998) Antiarrhythmic drugs and cardiac ion channels: mechanism of action. Prog Biophys Mol Biol 70:1–71

Chabbert C, Chambard JM, Sans A, Desmadryl G (2001) Three types of depolarization-activated potassium currents in acutely isolated mouse vestibular neurons. J Neurophysiol 85:1017–1026

Champeroux P, Guth BD, Markert M, Rast G (2013) Methods in Cardiovascular Safety Pharmacology. In: Vogel HG, Maas J, Hock FJ, Mayer D (eds) Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays, 2nd edn. Springer Berlin Heidelberg. p. 73–97

Colatsky TJ, Follmer CH, Starmer CF (1990) Channel specificity in antiarrhythmic drug action. Mechanism of potassium channel block and its role in suppressing and aggravating cardiac arrhythmias. Circulation 82:2235–2242

Escande D, Henry P (1993) Potassium channels as pharmacological targets in cardiovascular medicine. Eur Heart J 14(Suppl B):2–9

Gintant GA (1996) Two components of delayed rectifier current in canine atrium and ventricle. Does I_{Ks} play a role in the reverse rate dependence of class III agents? Circ Res 78:26–37

Gögelein H, Brüggemann A, Gerlach U, Brendel J, Busch AE (2000) Inhibition of I_{Ks}

channels by HMR 1556. Naunyn-Schmiedebergs Arch Pharmacol 362:480–488
Golding AL (1992) Maintenance of Xenopus laevis and oocyte injection. Methods Enzymol 207:266–279
Grissmer S, Nguyen AN, Aiyar J, Hanson DC, Mather RJ, Gutman GA, Karmilowicz MJ, Auperin DD, Chandy KG (1994) Pharmacological characterization of five cloned voltage-gated K^+ channels, types Kv1.1, 1.2, 1.3, 1.5, and 3.1, stably expressed in mammalian cell lines. Mol Pharmacol 45:1227–1234
Kaczorowski GJ, Garcia ML (1999) Pharmacology of voltage-gated and calcium-activates potassium channels. Curr Opin Chem Biol 3:448–458
Lei M, Brown HF (1996) Two components of the delayed rectifier potassium current, IK, in rabbit sinoatrial node cells. Exp Physiol 81:725–741
Longobardo M, Delpón E, Caballero R, Tamargo J, Valenzuela C (1998) Structural determinants of potency and stereoselective block of hKv1.5 channels induced by local anesthetics. Mol Pharmacol 54:162–169
Methfessel C, Witzemann V, Takahashi T, Mishina M, Numa S, Sakmann B (1986) Patch clamp measurements on Xenopus laevis oocytes: currents through endogenous channels and implanted acetylcholine receptor. Pflugers Arch 407:577–588
Moreno I, Caballero R, Ganzález T, Arias C, Valenzuela C, Iriepa I, Gálvez E, Tamargo J, Delpón E (2003) Effects of irbesartan on cloned potassium channels involved in human cardiac repolarization. J Pharmacol Exp Ther 304:862–873
Sanchez-Chapula JA (1999) Mechanisms of transient outward K^+ channel block by disopyramide. J Pharmacol Exp Ther 290:515–523
Sanchez-Chapula JA, Navarro-Polanco RA, Culberson C, Chen J, Sanguinetti MC (2002) Molecular determinants of voltage-dependent human ether-a-go-go related gene (HERG) K^+ channel block. J Biol Chem 277:23587–23595
Sanguinetti MC, Jurkiewicz NK (1990) Two components of cardiac delayed rectifier K^+ currents: differential sensitivity to block by class III antiarrhythmic agents. J Gen Physiol 96:195–215
Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MD (1996) Coassembly of KvLTQ1 and minK (IsK) proteins form cardiac IKs potassium channel. Nature 384:80–83
Suessbrich H, Busch A, Ecke D, Rizzo M, Waldegger S, Lang F, Szabo I, Lang HJ, Kunzelmann K, Greger R, Busch AE (1996) Specific blockade of slowly activating channels by chromanols – impact on the role of I_{sK} channels in epithelia. FEBS Lett 396:271–275
Suessbrich H, Busch AE, Scherz MW (1997) The pharmacology of cloned cardiac potassium channels. Ion Channel Modulators 2:432–439
Tagliatela M, Timmerman H, Annunziato L (2000) Cardiotoxic potential and CNS effects of first-generation antihistamines. Trends Pharmacol Sci 21:52–65
Vandenberg JI, Walker BD, Campbell TC (2001) HERG K^+ channels: friend and foe. Trends Pharmacol Sci 22:240–246
Wang Z, Fermini B, Nattel S (1994) Rapid and slow components of delayed rectifier current in human atrial myocytes. Cardiovasc Res 28:1540–1546
Zhou J, Angelli-Szafran CE, Bradley JA, Chen X, Koci BJ, Volberg WA, Sun Z, Cordes JS (2005) Novel potent human Ether-à-Go-Go-related gene (hERG) potassium channel enhancers and their in vitro antiarrhythmic activity. Mol Pharmacol 68:876–884

Studies on Kv1.5 Channel

Purpose and Rationale

Treatment of atrial fibrillation/flutter with available potassium channel blockers (class III antiarrhythmic agents which mainly block the delayed rectifier current I_{kr}) is associated with ventricular proarrhythmia. Prolongation of ventricular repolarization leads to early afterdepolarization from which torsades de pointes can evolve. Therefore,

blockade of a cardiac current of exclusive relevance in the atria is highly desirable as it is expected to be devoid of ventricular proarrhythmic effects. The ultrarapid delayed rectifier potassium current (I_{kur}) seems an ideal atrial antiarrhythmic target since it is found to contribute to the action potential in the atrium but not in the ventricle. The molecular correlate of the human cardiac ultrarapid delayed rectifier potassium current is the potassium channel Kv1.5, which therefore gained much interest (Li et al. 1996; Longobardo et al. 1998; Perchenet and Clément-Chomienne 2000; Caballero et al. 2000, 2001, 2004; Bachmann et al. 2001; Kobayashi et al. 2001; Matsuda et al. 2001; Choi et al. 2002; Moreno et al. 2003; Choe et al. 2003; Fedida et al. 2003; Godreau et al. 2002, 2003; Peukert et al. 2003, 2004; Plane et al. 2005).

For in vivo studies on atrial fibrillation, see sections "Experimental Atrial Fibrillation," "Atrial Fibrillation by Atrial Pacing in Dogs," "Atrial Fibrillation in Chronically Instrumented Goats," and "Influence on Ultrarapid Delayed Rectifier Potassium Current in Pigs," chapter "► Anti-Arrhythmic Activity".

Gögelein et al. (2004) studied the effects of the antiarrhythmic drug AVE0118 on cardiac ion channels.

Procedure

Molecular Biology and Cell Culture

Human Kv1.5 cDNA was subcloned into the eukaryotic expression vectors pcDNA3.1 and pcDNA3.1/zeo (Invitrogen, Groningen, the Netherlands), cDNA encoding human Kv4.3 long (Kv4.31; Dilks et al. 1999) was subcloned into pcDNA3.1, and the cDNA encoding human KChIP2 short (KChIP2.2; Decher et al. 2001) was subcloned into pcDNA3.1/zeo expression vector. Chinese hamster ovary (CHO) cells were transfected with either hKv1.5 or hKv4.3 and KChIP2.2 expression constructs. Transfection was carried out using lipofectamine (Life Technologies/Gibco BRL, Karlsruhe, Germany) according to the manufacturer's instructions. To boost Kv1.5 channel expression, CHO cells were consecutively transfected with both Kv1.5 expression constructs. Both hKv1.5 and hKv4.3 + hKChIP2.2 were stably expressed in CHO cells, which were maintained in ISCOVE's medium (Biochrom KG, Berlin, Germany), supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, 350 µg/ml Zeocin (Invitrogen), and 400 µg/ml G418 (PAA Laboratories). HERG, the potassium channel underlying I_{Kr} currents in human hearts, was cloned and transfected into CHO cells as described previously (Rampe et al. 1997). Cells used for patch clamping were seeded on glass or plastic coverslips 12–36 h before use.

Northern Blot Analysis of Kv1.5 in the Pig Heart and Cloning of Pig Kv1.5

Polyadenylated RNA was isolated from pig cardiac tissues with the Oligotex mRNA purification kit (Qiagen), and 10 µg per tissue was resolved by denaturing formaldehyde electrophoresis and blotted on a positively charged nylon membrane. The membrane was hybridized with a DIG-labeled riboprobe (DIG RNA labeling kit, Roche) encompassing the entire coding sequence of human Kv1.5 and exposed on a Lumi-Imager (Roche). The pig Kv1.5 was cloned by 5′-rapid amplification and 3′-rapid amplification of cDNA ends (RACE) reactions. An adapter-ligated, double-stranded cDNA library was prepared from pig heart mRNA with the Marathon cDNA Amplification Kit (Clontech). The 5′-RACE and 3′-RACE reactions were performed with oligonucleotide primers derived from a partial pig Kv1.5 nucleotide sequence (GenBank accession number AF348084). Overlapping cDNA clones were obtained by repeated reactions and the DNA sequence determined by automated DNA sequencing on both strands (ABI 310, PerkinElmer). A full-length cDNA clone was established by recombinant PCR. It encodes an open reading frame of 1,083 bp and a protein with 86 % overall sequence similarity to the human Kv1.5 protein. The sequence of the pig Kv1.5 cDNA was submitted to GenBank (accession number: AY635585).

For *Xenopus* oocyte expression, cDNAs encoding Kv1.5, Kv4.3, and KChip2.2 were cloned into the oocyte expression vector pSGEM (Villmann et al. 1997), and capped cRNA was

synthesized using the T7 mMessage mMachine kit (Ambion, Austin, Tex., USA).

Voltage-Clamp Experiments in Xenopus Oocytes

Handling and injection of *Xenopus* oocytes were performed according to Bachmann et al. (2001). Adult female *Xenopus laevis* frogs were anesthetized with 3-aminobenzoic acid ethyl ester solution (1 g/l) and intact ovary lobes were removed. The oocytes were defolliculated by treatment with 40 mg collagenase dissolved in 20 ml buffer (in mM: NaCl 82.5, KCl 2, $MgCl_2$ 1, HEPES 5, titrated to pH 7.5 with NaOH) for 120–150 min at 18 °C. Oocytes were injected with 50 nl cRNA using a microinjector (World Precision Instruments, Sarasota, Fla., USA). Oocytes were stored under gentle shaking at 18 °C in a buffer containing (in mM) NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, Na-pyruvate 2.5, theophylline 0.5, and gentamicin 50 µg/ml, titrated to pH7.5 with NaOH. They were used for experiments 1–3 days after injection.

Two-electrode voltage-clamp recordings were performed at room temperature in a medium containing (in mM) NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, and HEPES 5, at pH 7.5 with NaOH. Microelectrodes were pulled from filament borosilicate glass capillaries (Hilgenberg, Malsfeld, Germany) using a horizontal microelectrode puller (Zeitz, Augsburg, Germany). After filling with 3 M KCl, pipettes had a resistance of 0.3–1.3 MΩ. To activate hKv1.5 and hKv4.3 channels, oocytes were clamped from a holding potential of −80 mV to 40 mV for 500 ms. Data were recorded with a Turbo Tec 10CX amplifier (NPI, Tamm, Germany) using an ITC-16 interface (Instrutech Corporation, Long Island, USA) and the Pulse software (HEKA Elektronik, Lambrecht, Germany).

Patch-Clamp Experiments with CHO Cells

Cells expressing Kv1.5 or Kv4.3 plus KChIP2.2 were assayed using the standard whole-cell patch-clamp technique (Hamill et al. 1981). Cells were mechanically removed from the tissue culture flask and placed in a perfusion chamber with a solution containing (in mM) NaCl 140, KCl 4.7, $CaCl_2$ 2, $MgCl_2$ 1.1, and HEPES 10, at pH adjusted to 7.4 with NaOH. Patch pipettes were pulled from borosilicate glass capillaries and heat polished. After filling with (in mM) NaCl 10, KCl 120, EGTA 1, HEPES 10, and $MgCl_2$ 1.1 (pH 7.2 with potassium hydroxide, KOH), pipettes had resistances of 2–3 MΩ. Experiments were carried out at 36 ± 1 °C. For the recording of hKv1.5, voltage pulses of 450 ms duration were applied from the holding potential of −30 mV to +20 mV at a frequency of 1 Hz. For recording of the hKv4.3 +KChIP2.2, the holding potential was −50 mV and test pulses of 200 ms duration were applied to −10 mV at a frequency of 1 Hz. Data were recorded with an EPC-9 patch-clamp amplifier (HEKA Elektronik) and the Pulse software (HEKA Elektronik) and stored on a PC for later analysis. Series resistance was in the range of 4–9 MΩ and was compensated by 80 % by means of the EPC9's compensation circuit. The experiments were performed under continuous superfusion of the cells with solution heated to 36 ± 1 °C.

HERG channel currents were recorded at room temperature using the whole-cell configuration of the patch-clamp technique with an Axopatch 200B amplifier (Axon Instruments). Briefly, electrodes (3–6 MΩ resistance) were fashioned from TW150F glass capillary tubes (World Precision Instruments) and filled with pipette solution (in mM: potassium aspartate 120, KCl 20, Na_2ATP 4, HEPES 5, $MgCl_2$ 1, pH 7.2 adjusted with KOH). HERG currents were initiated by a positive voltage pulse (20 mV) followed by a negative pulse (−40 mV) and were recorded for off-line analyses. Once HERG current from a cell perfused with control external solution (in mM: NaCl 130, KCl 5, sodium acetate 2.8, $MgCl_2$ 1, HEPES 10, glucose 10, $CaCl_2$ 1 at pH 7.4 adjusted with NaOH) was stabilized, the cell was perfused with external solution containing the compound at a specific concentration for percentage inhibition. For each concentration from each cell, peak amplitude of the steady-state HERG tail current at −40 mV was measured. The peak amplitude for each concentration was compared with that for the control solution from the same cell and expressed as percent control.

Isolation of Porcine Atrial Myocytes

Male pigs weighing 15–30 kg of the German Landrace were anesthetized with pentobarbital exactly as described previously (Wirth and Knobloch 2001). After a left thoracotomy the lung was retracted, the pericardium was incised, and the heart was quickly removed and placed in oxygenated nominally Ca^{2+}-free Tyrode solution containing (in mM) NaCl 143, KCl 5.4, $MgCl_2$ 0.5, NaH_2PO_4 0.25, HEPES 5, and glucose 10, at pH adjusted to 7.2 with NaOH. The hearts were then mounted on a Langendorff apparatus and perfused via the left circumflex coronary artery with Tyrode solution (37 °C) with constant pressure (80 cmH_2O). All coronary vessels descending to the ventricular walls were ligated, ensuring sufficient perfusion of the left atrium. When the atrium was clear of blood and contraction had ceased (≈5 min), perfusion was continued with the same Tyrode solution, which now contained 0.015 mM $CaCl_2$ and 0.03 % collagenase (type CLS II, Biochrom KG, Berlin, Germany), until atrial tissue softened (≈20 min). Thereafter, left atrial tissue was cut into small pieces and mechanically dissociated by trituration. Cells were then washed with storage solution containing (in mM) L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 1, glucose 10, HEPES 10, and EGTA 2 (pH 7.2 with KOH) and filtered through a nylon mesh. The isolated cells were kept at room temperature in the storage solution.

Isolation of Guinea Pig Ventricular Myocytes

Ventricular myocytes were isolated by enzymatic digestion according to Gögelein et al. (1998). Dunkin–Hartley–Pirbright white guinea pigs (weight about 400 g) were sacrificed by cervical dislocation. The hearts were dissected and perfused retrogradely via the aorta at 37 °C with the same solutions as used for isolation of pig atrial myocytes.

Electrophysiological Recordings from Cardiac Myocytes

Whole-cell currents were recorded with an EPC-9 patch-clamp amplifier (HEKA Elektronik) as described above for CHO cells. A small aliquot of cell-containing solution was placed in a perfusion chamber, and after a brief period allowing for cell adhesion to the chamber, the cells were perfused with (in mM) NaCl 140, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.0, HEPES 10, and glucose 10, at pH adjusted to 7.4 with NaOH. Patch pipettes were pulled from borosilicate glass capillaries and heat polished. After filling with (in mM) KCl 130, $MgCl_2$ 1.2, HEPES 10, EGTA 10, K_2ATP 1, GTP 0.1, and phosphocreatine 5 (pH 7.2 with KOH), pipettes had a resistance of 2–3 MΩ. Series resistance was in the range of 6–12 MΩ and was compensated by 60–70 %. Offset voltages generated when the pipette was inserted in NaCl solution (1–5 mV) were zeroed before formation of the seal.

Effects of AVE0118 on the I_{KACh} were recorded from pig left atrial myocytes by applying voltage pulses of 500 ms duration from the holding potential of −80 mV to −100 mV. Carbachol (10 μM) was added in order to evoke the I_{KACh}. After stabilization of the I_{KACh} (3 min), AVE0118 was added in increasing concentrations in the continuous presence of carbachol. The current was measured at the end of the pulse after 3 min of incubation at each concentration, and inhibition of the carbachol-activated current was calculated. In some experiments, AVE0118 was washed out before application of the next higher concentration.

Also the L-type Ca^{2+} current was investigated in pig left atrial cells. In these experiments, KCl in the pipette was replaced by CsCl, and voltage pulses of 300 ms duration were applied from the potential of −40 mV to 0 mV. Possible effects of AVE0118 on the currents I_{K1}, I_{Ks}, I_{Kr}, and I_{KATP} were investigated in guinea pig ventricular myocytes. I_{K1} currents were recorded by a voltage step from −80 mV to −120 mV lasting for 200 ms. When I_{Ks} and I_{Kr} currents were recorded, 1 μM nisoldipine was added to the bath to block the L-type Ca^{2+} current. I_{Ks} was assessed by voltage pulse to +60 mV for 3 s, starting from −40 mV. I_{Kr} was evaluated as the tail current evoked by a voltage pulse from −10 mV to −40 mV. I_{KATP} was evoked by adding 1 μM rilmakalim (Krause et al. 1995) to the bath and

by applying voltage ramps from −130 mV to +80 mV for 500 ms. The rilmakalim-activated current was recorded at the potential 0 mV. All patch-clamp experiments were performed under continuous superfusion of the cells with solution heated to 36 ± 1 °C.

Evaluation

All averaged data are presented as the mean ±SEM. The Student's *t*-test was used to determine the significance of paired or unpaired observations. Differences were considered significant at $P < 0.05$. The values for half-maximal inhibition (IC_{50}) and the Hill coefficient were calculated by fitting the data points of the concentration–response curves to the logistic function:

$$f(x) = (a - d)/[1 + (x/c)^n] + d$$

where a represents the plateau value at low drug concentration, d the plateau value at high drug concentration, c the IC_{50} value, and n the Hill coefficient. The curve fitting and the Student's *t*-test were performed with the computer program *Sigma-Plot* 5.0.

References and Further Reading

Bachmann A, Gutcher I, Kopp K, Brendel J, Bosch RF, Busch AE, Gögelein H (2001) Characterization of a novel Kv1.5 channel blocker in Xenopus oocytes, CHO cells, and human cardiomyocytes. Naunyn-Schmiedebergs Arch Pharmacol 364:472–478

Caballero R, Delpón E, Valenzuela C, Longobardo M, Tamargo J (2000) Losartan and its metabolite E3174 modify cardiac delayed rectifier K^+ currents. Circulation 101:1199–1205

Caballero R, Delpón E, Valenzuela C, Longobardo M, González T, Tamagro J (2001) Direct effects of candesartan and eprosartan in human cloned potassium channels involved in cardiac repolarization. Mol Pharmacol 59:825–836

Caballero R, Gómez R, Núòez L, Moreno I, Tamargo J, Delpón E (2004) Diltiazem inhibits hKv1.5 and Kv4.3 currents in therapeutic concentrations. Cardiovasc Res 64:457–466

Choe H, Lee YK, Lee YT, Choe H, Ko SH, Joo CU, Kim MH, Kim GS, Eun JS, Kim JH, Chae SW, Kwak YG (2003) Papaverine blocks hKv1.5 channel current und human atrial ultrarapid delayed rectifier K^+ currents. J Pharmacol Exp Ther 304:706–712

Choi BH, Choi JS, Rhie DJ, Yoon SH, Min DS, Jo YH, Kim MS, Hahn SJ (2002) Direct inhibition of the cloned Kv1.5 channel by AG-1478, a tyrosine kinase inhibitor. Am J Physiol 282: C1461–C1468

Decher N, Uyguner O, Scherer CR, Karaman B, Yüksel-Apak M, Busch AE, Steinmeyer K, Wollnik B (2001) hKChIP2 is a functional modifier of hKv4.3 potassium channels: cloning and expression of a short hKChIP2 splice variant. Cardiovasc Res 52:255–264

Dilks D, Ling H-P, Cockett M, Sokol P, Numann R (1999) Cloning and expression of the human Kv4.3 potassium channel. J Neurophysiol 81:1974–1977

Fedida D, Eldstrom J, Hesketh C, Lamorgese M, Castel L, Steele DF, van Wagoner DR (2003) Kv1.5 is an important component of repolarizing K^+ current in canine atrial myocytes. Circ Res 93:744–751

Godreau D, Vranckx R, Hatem SN (2002) Mechanism of action of antiarrhythmic agent bertosamil on hKv1.5 channels and outward current in human atrial myocytes. J Pharmacol Exp Ther 300:612–620

Godreau D, Vranckx R, Maguy A, Goyenvalle C, Hatem SN (2003) Different isoforms of synapse-associated protein, SAP97, are expressed in the heart and have distinct effects on the voltage-gated K^+ channel Kv1.5. J Biol Chem 278:47046–47052

Gögelein H, Hartung J, Englert HC, Schölkens BA (1998) HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. I. Effects on cardiomyocytes, coronary flow and pancreatic β-cells. J Pharmacol Exp Ther 286:1453–1464

Gögelein H, Brendel J, Steinmeyer K, Strübing C, Picard N, Rampe D, Kopp K, Busch AE, Bleich M (2004) Effects of the antiarrhythmic

drug AVE0118 on cardiac ion channels. Naunyn-Schmiedebergs Arch Pharmacol 370:183–192

Hamill OP, Marty M, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85–100

Kobayashi S, Reien Y, Ogura T, Saito T, Masuda Y, Nakaya H (2001) Inhibitory effect of bepridil on hKv1.5 channel current; comparison with amiodarone and E-4031. Eur J Pharmacol 430:149–157

Krause E, Englert H, Gögelein H (1995) Adenosine triphosphate-dependent K currents activated by metabolic inhibition in rat ventricular myocytes differ from those elicited by the channel opener rilmakalim. Pflügers Arch 429:625–635

Li GR, Feng J, Wang Z, Fermine B, Nattel S (1996) Adrenergic modulation of ultrarapid rectifier K^+ current in human atrial myocytes. Circ Res 78:903–915

Longobardo M, González T, Navarro-Polanco R, Calballero R, Delpón E, Tamargo J, Snyders DJ, Tamkum MM, Valenzuela C (2000) Effects of a quaternary bupivacaine derivative on delayed rectifier K^+ currents. Br J Pharmacol 130:391–401

Matsuda T, Masumiya H, Tanaka N, Yamashita T, Tsuruzoe N, Tanaka Y, Tanaka H, Shigenoba K (2001) Inhibition by a novel anti-arrhythmic agent, NIP-142, of cloned human cardiac K^+ channel Kv1.5 current. Life Sci 68:2017–2024

Moreno I, Caballero R, González T, Arias C, Valenzuela C, Iriepa I, Gálvez E, Tamargo J, Delpón E (2003) Effects of irbesartan on cloned potassium channels involved in human cardiac repolarization. J Pharmacol Exp Ther 304:862–873

Perchenet L, Clément-Chomienne O (2000) Characterization of the mibefradil block of the human heart delayed rectifier hKv1.5. J Pharmacol Exp Ther 295:771–778

Peukert S, Brendel J, Pirad B, Bruggemann A, Below P, Kleemann HW, Hemmerle H, Schmidt W (2003) Identification, synthesis, and activity of novel blockers of the voltage-gated potassium channel Kv1.5. J Med Chem 46:486–498

Peukert S, Brendel J, Pirard B, Strübing C, Kleemann HW, Böhme T, Hemmerle H (2004) Pharmacophore-based search, synthesis, and biological evaluation of anthranilic amides as novel blockers of the Kv1.5 channel. Bioorg Med Chem Lett 14:2823–2827

Plane F, Johnson R, Kerr P, Wiehler W, Thorneloe K, Ishii K, Chen T, Cole W (2005) Heteromultimeric Kv1 channels contribute to myogenic control of arterial diameter. Circ Res 96:216–224

Rampe D, Roy ML, Dennis A, Brown AM (1997) A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. FEBS Lett 417:28–32

Villmann C, Bull L, Hollmann M (1997) Kainate binding proteins possess functional ion channel domains. J Neurosci 17:7634–7643

Wirth KJ, Knobloch K (2001) Differential effects of dofetilide, amiodarone, and class Ic drugs on left and right atrial refractoriness and left atrial vulnerability in pigs. Naunyn-Schmiedebergs Arch Pharmacol 363:166–174

Voltage-Clamp Studies on Calcium Channels

Purpose and Rationale

Calcium influx through voltage-gated Ca^{2+} channels mediates a range of cytoplasmatic responses, including muscle contraction, release of neurotransmitters, Ca^{2+}-dependent gene transcription, and the regulation of neuronal excitability, and has been reviewed by several authors (Augustine et al. 1987; Bean 1989; Miller 1987; Zamponi 1997; Snutch et al. 2001). In addition to their normal physiological function, Ca^{2+} channels as calcium antagonists are also implicated in a number of human disorders (see also "▶ Calcium Uptake Inhibition Activity").

Using patch-clamp techniques, the structure and regulation of voltage-gated Ca^{2+} channels has been studied by many authors (Sculptoreanu et al. 1993; Peterson et al. 1997; Catterall 2000).

Berjukow et al. (2000) analyzed the role of the inactivated channel conformation in molecular mechanism of Ca^{2+} channel block by a dihydropyridine derivative in L-type channel constructs and mutants in *Xenopus* oocytes and described the electrophysiological evaluation.

Procedure

Inward barium currents (I_{Ba}) are studied with two-microelectrode voltage clamp of *Xenopus* oocytes 2–7 days after microinjection of approximately equimolar cRNA mixtures of constructs of L-channel mutants. All experiments are carried out at room temperature in a bath solution with the following composition: 40 mM $Ba(OH)_2$, 50 mM NaOH, 5 mM HEPES, and 2 mM CsOH (pH adjusted to 7.4 with methanesulfonic acid). Voltage-recording and current-injecting microelectrodes are filled with 2.8 M CsCl, 0.2 M CsOH, 10 mM EGTA, and 10 mM HEPES (pH 7.4) with resistances of 0.3–2 MΩ. Resting channel block is estimated as peak I_{Ba} inhibition during 100-ms test pulses from −80 to 20 mV at a frequency of 0.033 Hz until steady state is reached. The dose–response curves of I_{Ba} inhibition were fitted using the Hill equation:

$$\frac{I_{\text{Ba, drug}}}{I_{\text{Ba, control}}}(\%) = \frac{100 - A}{1 + \left(\frac{C}{IC_{50}}\right)^{nH}} + A$$

where IC_{50} is the concentration at which I_{Ba} inhibition is half maximal, C is the applied drug concentration, A is the fraction of I_{Ba} that is not blocked, and nH is the Hill coefficient.

Recovery from inactivation is studied at a holding potential of −80 mV after depolarizing Ca^{2+} channels during a 3-s prepulse to 20 mV by applying 30-ms test pulses (to 20 mV) at various time intervals after the conditioning prepulse. Peak I_{Ba} values are normalized to the peak current measured during the prepulse, and the time course of I_{Ba} recovery from inactivation is fitted to a mono- or biexponential function:

$$I_{\text{Ba, recovery}} = A \times \exp\left(\frac{-t}{\tau_{\text{fast}}}\right) + B \times \exp\left(\frac{-t}{\tau_{\text{slow}}}\right) + C$$

Voltage dependence of inactivation under quasi-steady-state conditions is measured using a multistep protocol to account for rundown (less than 10 %). A control test pulse (50 ms to 20 mV) is followed by a 1.5-s step to −100 mV followed by a 30-s conditioning step, a 4-ms step to −100 mV, and a subsequent test pulse to 20 mV (corresponding to the peak potential of the I–V curves).

Inactivation during the 30 s conditioning pulse is calculated as follows:

$$I_{\text{Ba, inactivation}} = \frac{I_{\text{Ba, test}}(20\text{mV})}{I_{\text{Ba, control}}(20\text{mV})}$$

The pulse sequence is applied every 3 min from a holding potential of −100 mV. Inactivation curves are drawn according to the following Boltzmann equation:

$$I_{\text{Ba, inactivation}} = I_{SS} + (1 - I_{SS})\left(1 + \exp\left(\frac{V - V_{0.5}}{k}\right)\right)$$

where V is the membrane potential, $V_{0.5}$ is the midpoint voltage, k is the slope factor, and I_{SS} is the fraction of non-inactivating current.

Steady-state inactivation of the mutate channels at −80 mV is estimated by shifting the membrane holding potential from −80 to −100 mV. Subsequent monitoring of the corresponding changes in I_{Ba} amplitudes until steady state reveals the fraction of Ca^{2+} channels in the inactivated state at −80 mV. Steady-state inactivation of different L-type channel constructs at −30 mV is estimated by fitting time course of current inactivation to a biexponential function.

The I_{Ba} inactivation time constants are estimated by fitting the I_{Ba} decay to a mono- or biexponential function.

Evaluation

Data are given as the means ±SE. Statistical significance is calculated according to Student's unpaired *t*-test.

Modifications of the Method

Besides *Xenopus* oocytes (Waard and Campell 1995; Hering et al. 1997; Kraus et al. 1998), several other cell types and constructs, such as CHO cells (Sculptoreanu et al. 1993; Stephens et al. 1997); HEK293 (human embryonic kidney) cells (Lacinová et al. 1999); tsA-201 cells, a subclone of HEK293 (Peterson et al. 1997; McHugh et al. 2000); cardiac myocytes from rats (Scamps et al. 1990; Tohse et al. 1992; Gomez et al. 1994) and rabbits (Xu et al. 2000); isolated atrial myocytes from failing and non-failing human hearts (Cheng et al. 1996); skeletal muscle myotubes from mice and rabbits (Johnson et al. 1994); myocytes of guinea pig mesenteric artery (Morita et al. 1999); dendrites from rat pyramidal and olfactory bulb neurons (Markram and Sakmann 1994; Stuart and Spruston 1995; Koester and Sakmann 1998; Margie et al. 2001); and rat amygdala neurons (Foehring and Srcoggs 1994; Young et al. 2001), were used to study the function of calcium channels.

Using the whole-cell variation of the patch-clamp technique, Yang et al. (2000) studied cellular T-type and L-type calcium channel currents in mouse neuroblastoma N1E115 cells. The cells were cultured in Dulbecco's Modified Eagle's Medium containing 10 % fetal bovine serum at 37 °C in a humidified atmosphere of 5 % CO_2 in air. The medium was changed every 3–4 days. After mechanical agitation, 3×10^4 cells were replanted in 35-mm tissue culture dishes containing 4 ml of bath solution. After cell attachment, the dish was mounted on the stage of an inverted phase-contrast microscope for Ca^{2+} channel current recording. These cells expressed predominantly T channel currents. In experiments where L channels were specifically sought, the cells were grown and maintained at confluence for 3–4 weeks under the same culture conditions with the addition of 2 % dimethyl sulfoxide (Narahash et al. 1987). Three to 5 days before use, the cells were replanted with the same medium. These cells expressed predominantly L channel currents. A small number of these cells also expressed T channel currents. Hence, cells were selected so that at a holding potential of −40 mV, the T channel component was very small and the inward current measured was conducted predominantly by L channels.

By using whole-cell and perforated patch-clamp techniques, Wu et al. (2000) showed that mifrabidile, a non-dihydropyridine compound, has an inhibitory effect on both T- and L-type Ca^{2+} currents in pancreatic β-cells.

References and Further Reading

Augustine GJ, Charlton MP, Smith RJ (1987) Calcium action in synaptic transmitter release. Annu Rev Neurosci 10:633–693

Bean BP (1989) Classes of calcium channels in vertebrate cells. Annu Rev Physiol 51:376–384

Berjukom S, Marksteiner R, Gapp F, Sinneger MJ, Hering S (2000) Molecular mechanism of calcium channel block by isradipine. Role of a drug-induced inactivated channel configuration. J Biol Chem 275:22114–22120

Catterall WA (2000) Structure and regulation of voltage-gated Ca^{2+} channels. Ann Rev Cell Dev Biol 16:521–555

Cheng T-H, Lee F-Y, Wei J, Lin C-I (1996) Comparison of calcium-current in isolated atrial myocytes from failing and nonfailing human hearts. Mol Cell Biochem 157:157–162

Foehring RC, Srcoggs RS (1994) Multiple high-threshold calcium currents in acutely isolated rat amygdaloid pyramidal cells. J Neurophysiol 71:433–436

Gomez JP, Potreau D, Branka JE, Raymond G (1994) Developmental changes in Ca^{2+} currents from newborn rat cardiomyocytes in primary culture. Pflugers Arch 428:214–249

Hering S, Aczél S, Klaus RL, Berjukow S, Striessnig J, Timin EN (1997) Molecular

mechanism of use-dependent calcium channel block by phenylalkylamines: role of inactivation. Proc Natl Acad Sci U S A 94:13323–13328

Hockerman GH, Peterson BZ, Sharp E, Tanada TN, Scheuer T, Catterall WA (1997) Construction of a high-affinity receptor site for dihydropyridine agonists and antagonists by single amino acid substitution in a non-L-type Ca^{2+} channel. Proc Natl Acad Sci USA 94:14906–14911

Johnson BD, Scheuer T, Catterall WA (1994) Voltage-dependent potentiation of L-type Ca^{2+} channels in skeletal muscles cells requires anchored cAMP-dependent protein kinase. Proc Natl Acad Sci 91:11492–11496

Koester HJ, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagation action potentials and subthreshold excitatory potentials. Proc Natl Acad Sci U S A 95:9596–9601

Kraus RL, Hering S, Grabner M, Ostler D, Striessnig J (1998) Molecular mechanisms of diltiazem interaction with L-type Ca^{2+} channels. J Biol Chem 273:27205–27212

Lacinová L, An HR, Xia J, Ito H, Klugbauer N, Triggle E, Hofmann F, Kass RS (1999) Distinction in the molecular determinants of charged and neutral dihydropyridine block of L-type calcium channels. J Pharmacol Exp Ther 289:1472–1479

Lacinová L, Klugbauer N, Hofmann F (2000) State- and isoform-dependent interaction of isradipine with the α_{1C} L-type calcium channel. Pflügers Arch Eur J Physiol 440:50–60

Margrie TW, Sakmann B, Urban NN (2001) Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb. Proc Natl Acad Sci U S A 98:319–324

Markram H, Sakmann B (1994) Calcium transients in dendrites of neocortical neurons evoked by single subthreshold excitatory postsynaptic potentials via low-voltage-activated calcium channels. Proc Natl Acad Sci U S A 91:5207–5211

McHugh D, Sharp EM, Scheuer T, Catterall WA (2000) Inhibition of L-type calcium channels by protein kinase C phosphorylation of two sites in the N-terminal domain. Proc Natl Acad Sci U S A 97:12334–12338

Miller RJ (1987) Multiple calcium channels and neuronal function. Science 235:46–52

Morita H, Cousins H, Inoue H, Ito Y, Inoue R (1999) Predominant distribution of nifedipine-insensitive, high voltage-activated Ca^{2+} channels in the terminal mesenteric artery of guinea pig. Circ Res 85:596–605

Narahash T, Tsunoo A, Yoshii M (1987) Characterization of two types of calcium channels in mouse neuroblastoma cells. J Physiol 38:231–249

Peterson BZ, Johnson BD, Hockerman GH, Acheson M, Scheuer T, Catterall WA (1997) Analysis of the dihydropyridine receptor site of L-type calcium channels by alanine-scanning mutagenesis. J Biol Chem 272:18752–18758

Scamps F, Mayoux E, Charlemagne D, Vassort G (1990) Calcium current in single cells isolated from normal and hypertrophied rat heart. Circ Res 67:199–208

Sculptoreanu A, Rotman E, Takahasi M, Scheuer T, Catterall WA (1993) Voltage-dependent potentiation of the activity of cardiac L-type calcium channel α1 subunits due to phosphorylation by cAMP-dependent protein kinase. Proc Natl Acad Sci U S A 90:10135–10139

Snutch TP, Sutton KG, Zamponi GW (2001) Voltage-dependent calcium channels – beyond dihydropyridine antagonists. Curr Opin Pharmacol 1:11–16

Stephens GJ, Page KM, Burley JR, Berrow NS, Dolphin AC (1997) Functional expression of brain cloned α1E calcium channels in COS-7 cells. Pflugers Arch 433:525–532

Stuart G, Spruston N (1995) Probing dendritic function with patch pipettes. Curr Opin Neurobiol 5:389–394

Tohse N, Masuda H, Sperelakis N (1992) Novel isoform of Ca^{2+} channel in rat

fetal cardiomyocytes. J Physiol (London) 451:295–306
Waard MD, Campell KP (1995) Subunit regulation of the neuronal α_{1A} Ca^{2+} channel expressed in Xenopus oocytes. J Physiol (London) 485:619–634
Wu S, Zhang M, Vest PA, Bhattacharjee A, Liu L, Li M (2000) A mifrabidile metabolite is a potent intracellular blocker of L-type Ca^{2+} currents in pancreatic β-cells. J Pharmacol Exp Ther 292:939–943
Xu X, Rials SJ, Wu Y, Liu T, Marinchak RA, Kowey PR (2000) Effects of captopril treatment of renovascular hypertension on β-adrenergic modulation of L-type Ca^{2+} current. J Pharmacol Exp Ther 292:196–200
Yang JC, Shan J, Ng KF, Pang P (2000) Morphine and methadone have different effects on calcium channel currents in neuroblastoma cells. Brain Res 870:199–203
Young C, Huang Y-C, Lin C-H, Shen Y-Z, Gean P-W (2001) Selective enhancement of L-type calcium currents by corticotropin in acutely isolated rat amygdala neurons. Mol Pharmacol 59:604–611
Zamponi GW (1997) Antagonist sites of voltage-dependent calcium channels. Drug Dev Res 42:131–143

Patch-Clamp Studies on Chloride Channels

Purpose and Rationale

Cl^- channels are a large, ubiquitous, and highly diverse group of ion channels involved in many physiological key processes including regulation of electrical excitability, muscle contraction, secretion, and sensory signal transduction. Cl^- channels belong to several distinct families characterized in detail: voltage-gated Cl^- channels, the cAMP-regulated channel CFTR (cystic fibrosis transmembrane conductance regulator), ligand-gated Cl^- channels that open upon binding to the neurotransmitters GABA or glycine, and Cl^- channels that are regulated by the cytosolic Ca^{2+} concentration (Jentsch and Günther 1997; Frings et al. 2000).

Cliff and Frizel (1990) studied the cAMP- and Ca^{2+}-activated secretory Cl^- conductances in the Cl^--secreting colonic tumor epithelial cell line T84 using the whole-cell voltage-clamp technique.

Procedure

T84 cells are used 1–3 days after plating on collagen-coating coverslips. The cells are maintained at 37 °C. At this temperature, the responsiveness of the cells to secretagogues, particularly to cAMP-dependent agonists, is improved. Increases in Cl^- and K^+ conductances are the major electrical events during stimulation of Cl^- secretion. Accordingly, bath–pipette ion gradients are chosen so that transmembrane Cl^- and K^+ currents can be monitored independently at clamp voltages equal to the reversal potentials of these ions. The pipette solution is 115 mM KCl, 25 mM *N*-methyl-D-glucamine (NMDG) glutamate, 0.5 mM EGTA, 0.19 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM Na_2ATP, 0.05 mM Na_3GPT, and 5 mM HEPES, at pH 7.2. The bath solution is 115 mM NaCl, 40 mM NMDG glutamate, 5 mM potassium glutamate, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM HEPES, at pH 7.2. Bath Na^+ and Cl^- concentrations are reduced by substituting NMDG chloride or sodium glutamate for NaCl. When Na^+- and K^+-free solutions are used, Na^+ and K^+ are replaced by $NMDG^+$, and Cl^- is reduced by replacing Cl^- by glutamate.

During whole-cell recording, the membrane potential is clamped alternately to three different voltages, each for 500-ms duration. Computer-controlled voltage-clamp protocols are used to generate current–voltage (I–V) relations when the transmembrane currents are relatively stable by stepping the clamp voltage between −100 mV and +100 mV at 20 mV intervals.

Test drugs (e.g., 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate, A23187, forskolin, or ionomycin) are solubilized in stock solutions (ethanol of DMSO) and diluted.

Evaluation

Instantaneous relations are constructed from currents recorded 6 ms after a voltage step.

Modifications of the Method

Maertens et al. (2000) used the whole-cell patch-clamp technique to study the effect of an antimalarial drug on the volume-regulated anion channel (VRAC) in cultured bovine pulmonary artery endothelial cells. They also examined the effects on other Cl^- channels, i.e., the Ca^{2+}-activated Cl^- channel and the cystic fibrosis transmembrane conductance regulator, to assess the specificity for VRAC.

Pusch et al. (2000) characterized chloride channels belonging to the ClC family. Chiral clofibric acid derivates were tested on the human ClC-1 channel, a skeletal muscle chloride channel, after heterologous expression in *Xenopus laevis* oocytes by means of two-microelectrode voltage-clamp recordings.

References and Further Reading

Cliff WH, Frizzell RA (1990) Separate Cl- conductances activated by cAMP and Ca^{2+} in Cl(-)-secreting epithelial cells. Proc Natl Acad Sci U S A 87(13):4956–60

Frings S, Reuter D, Kleene SJ (2000) Neuronal Ca^{2+} activated Cl^- channels – homing in on an elusive channel species. Prog Neurobiol 60:247–289

Jentsch TJ, Günther W (1997) Chloride channels: an emerging molecular picture. Bioessays 19:117–126

Maertens C, Wie L, Droogmans G, Nilius B (2000) Inhibition of volume-regulated and calcium-activated chloride channels by the antimalarial mefloquine. J Pharmacol Exp Ther 295:29–36

Pusch M, Liantonio A, Bertorello L, Accardi A, de Lucca A, Pierno S, Tortorella V, Camerino DC (2000) Pharmacological characterization of chloride channels belonging to the ClC family by the use of chiral clofibric acid derivates. Mol Pharmacol 58:498–507

Inhibition of Hyperpolarization-Activated Channels

Purpose and Rationale

The hyperpolarization-activated cation currents (termed I_f, I_h, or I_q) play a key role in the initiation of cardiac and neuronal pacemaker depolarizations. Unlike most voltage-gated channels, they are activated by hyperpolarizing voltage steps to potentials negative to −60 mV, near the resting potential of most cells. This property earned them the designation of I_f for "funny" or I_q for "queer." The funny current, or pacemaker (I_f) current, was first described in cardiac pacemaker cells of the mammalian sinoatrial node as a current that slowly activates on hyperpolarization at voltages in the diastolic voltage range and contributes to the generation of cardiac rhythmic activity and to its control by sympathetic and parasympathetic innervations (DiFrancesco et al. 1986; Accili et al. 1997, 2002; Robinson and Siegelbaum 2003; Baruscotti et al. 2005). In sinoatrial cells, f-channels are modulated by cAMP independently of phosphorylation, through a mechanism involving direct interaction of cAMP with the intracellular side of the channels (DiFrancesco and Tortora 1991; Bois et al. 1996). A significant advancement in the study of molecular properties of pacemaker channels was achieved when a new family of channels was cloned, the HCN (hyperpolarization-activated, cyclic nucleotide-gated) channels (Ishii et al. 1999; Kaupp and Seifert 2001; Biel et al. 2002; Macri et al. 2002). The HCN family is related to the cyclic nucleotide-gated channel and *eag* potassium channel family and belongs to the superfamily of voltage-gated cation channels. HCN channels are characterized by six membrane-spanning segments (S1–S6) including voltage-sensing (S4) and pore (between S5 and S6) regions. In the C-terminal region, they contain a consensus sequence for binding of cyclic nucleotides. In the heart, neurotransmitter-induced control of cardiac rhythm is mediated by I_f through its

second-messenger cAMP, whose synthesis is stimulated and inhibited by β-adrenoceptor and muscarinic agonists, respectively.

Inhibition of the I_f channel was recommended for induction of bradycardia and treatment of coronary disease (Thollon et al. 1994, 1997; Simon et al. 1995; Bois et al. 1996; Deplon et al. 1996; Acilli et al. 1997; Rocchetti et al. 1999; Monnet et al. 2001, 2004; Bucchi et al. 2002; Cerbai et al. 2003; Rigg et al. 2003; Vilaine et al. 2003; Albaladejo et al. 2004; Colin et al. 2004; DiFrancesco and Camm 2004; Moreno 2004; Mulder et al. 2004; Vilaine 2004; Chatelier et al. 2005; Leoni et al. 2005; Romanelli et al. 2005; Schipke et al. 2006).

Romanelli et al. (2005) reported the design, synthesis, and preliminary biological evaluation of zatebradine analogues as potential blockers of hyperpolarization-activated current, and Chatelier et al. (2005) described that a calmodulin antagonist directly inhibits f-type current in rabbit sinoatrial cells.

Procedure

Sinoatrial Cell Isolation

Sinoatrial node myocytes of the rabbit were isolated (DiFrancesco et al. 1986). Cells were allowed to settle in Petri dishes and were superfused with normal Tyrode solution containing (in mM) NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 5.5, and HEPES–NaOH 5, at pH 7.4.

Electrophysiology

In macro-patch experiments the temperature was kept at 27–28 °C and the patch pipette solution contained (in mM) NaCl 70, KCl 70, $CaCl_2$ 1.8, $MgCl_2$ 1, $BaCl_2$ 1, $MnCl_2$ 2, and HEPES–KOH 5, at pH 7.4. The control solution perfusing the intracellular side of the membrane patches contained (in mM) potassium aspartate 130, NaCl 10, $CaCl_2$ 2, EGTA 5, and HEPES–KOH 10, at pH 7.2, pCa = 7. In some experiments, the calcium concentration of the bath solution was reduced to 0.1 nM according to the calculation of Fabiato and Fabiato (1979) and the correction of Tsien and Rink (1980).

Macro-patches containing hundreds of f-channels were formed using a large-tipped pipette (0.5–2 MΩ) (DiFrancesco and Tortora 1991). The test compound or calmodulin (Calbiochem) was dissolved in either distilled water and ethanol (50/50) or distilled water, respectively, divided into aliquots, and stored at −20 °C until use. Ethanol was added to control solutions at the same concentration used in test solutions (lower than 0.1 %).

Evaluation

The time course of macro-patch I_f under the influence of the modifying compounds was recorded by applying hyperpolarizing steps of 3 s duration at a frequency of 1/15 Hz. At steady state, the voltage dependence of I_f was described by the equation $I_f(E) = g_f(E) \cdot (E - vE_f) = g_{fmax} \cdot y_\infty(E) \cdot (E - E_f)$, where g_f is the conductance, g_{fmax} the fully activated conductance, $y\infty$ (E) the steady-state activation parameter, and E_f the reversal potential (DiFrancesco and Noble 1985). Steady-state current–voltage (I–V) curves were measured by applying 1-min-long hyperpolarizing voltage ramps with a rate of −115 mV/min from a holding potential of −35 mV. Conductance–voltage (g_f/E) relations were then obtained from the above equation as ratios between steady-state I–V curves (i_f/E) and $E - E_f$, where E_f was set to −12.24 mV (DiFrancesco and Mangoni 1994). Conductance curves were fitted by Boltzmann function, $g_f(E) = g_{fmax} \cdot y_\infty(E) = g_{fmax} \cdot 1/[1 + \exp(E - E_{1/2})/p]$, where $E_{1/2}$ is the half-maximal voltage of activation and p is the inverse-slope factor. This allowed estimation of the shifts of the voltage dependence of conductance (i.e., of the activation parameter y_∞) measured as changes in $E_{1/2}$. Shifts of the I_f activation curve caused by cAMP were also determined by a quicker method not requiring measurement of the conductance–voltage relation (Accili and DiFrancesco 1996). Shifts were obtained by applying hyperpolarizing steps from −35 mV to near the midpoint of the I_f activation curve and adjusting the holding potential (−35 mV in the control solution) until the cAMP-induced change

in I_f was compensated and the control I_f magnitude fully restored. Since the compensation involved a change of the test voltage (from E to $E + s_m$, where s_m is the measured displacement of the holding potential in mV), a correction was introduced to obtain the shift of the activation curve (s, mV), according to the relation: $s = s_m \cdot [+(y_\infty/(dy_\infty/dE)]/(E - E_f)$.

When comparing different sets of data, statistical analysis was performed with either the Student's t-test or analysis of variance (ANOVA). Values of $P < 0.05$ were considered significant. Statistical data were given as mean ±SEM values.

References and Further Reading

Albaladejo P, Challande P, Kakou A, Benetos A, Labat C, Louis H, Safar ME, Lacolley P (2004) Selective reduction of heart rate by ivabradine: effect on the visco-elastic arterial properties in rats. J Hypertens 22:1739–1745

Accili EA, DiFrancesco D (1996) Inhibition of the hyperpolarization-activated current (if) of rabbit SA node myocytes by niflumic acid. Pflugers Arch 431:757–762

Accili EA, Robinson RB, DiFrancesco D (1997) Properties and modulation of I_f in newborn versus adult SA node. Am J Physiol 272: H1549–H1552

Accili EA, Proenza C, Baruscotti M, DiFrancesco D (2002) From funny current to HCN channels: 20 years of excitation. News Physiol Sci 17:32–37

Baruscotti M, Bucchi A, DiFrancesco D (2005) Physiology and pharmacology of the cardiac pacemaker ("funny") current. Pharmacol Ther 107:59–79

Biel M, Schneider A, Wahl C (2002) Cardiac HCN channels: structure, function, and modulation. Trends Cardiovasc Med 12:206–2134

Bois P, Bescond J, Renaudon B, Lenfant J (1996) Mode of action of bradycardic agent, S-16257, on ionic currents of rabbit sinoatrial node cells. Br J Pharmacol 118:1051–1057

Bucchi A, Baruscotti M, DiFrancesco D (2002) Current-dependent block of rabbit sino-atrial node I_f channels by Ivabradine. J Gen Physiol 120:1–13

Cerbai E, de Paoli P, Sartiani L, Lonardo G, Mugelli A (2003) Treatment with Irbesartan counteracts the functional remodeling of ventricular myocytes from hypertensive rats. J Cardiovasc Pharmacol 41:804–812

Chatelier A, Renaudon B, Bescond J, El Chemaly A, Demion M, Bois P (2005) Calmodulin antagonist W7 directly inhibits f-type current in rabbit sino-atrial cells. Eur J Pharmacol 521:29–33

Colin P, Ghaleb B, Monnet X, Hittinger L, Berdeaux A (2004) Effect of graded heart rate reduction with Ivabradine on myocardial oxygen consumption and diastolic time in exercising dogs. J Pharmacol Exp Ther 308:236–240

Deplon E, Valenzuela C, Perez O, Franqueza L, Gay P, Snyders DJ, Tamargo J (1996) Mechanisms of block of human cloned potassium channel by the enantiomers of a new bradycardic agent: S-16257–2 and S-1620–2. Br J Pharmacol 117:1293–1301

DiFrancesco D, Camm JA (2004) Heart rate lowering by specific and selective I_f current inhibition with Ivabradine. Drugs 64:1757–1765

DiFrancesco D, Mangoni M (1994) Modulation of single hyperpolarization-activated channels i_f by cAMP in the rabbit sino-atrial node. J Physiol (London) 474:473–482

DiFrancesco D, Noble D (1985) A model of cardiac electrical activity incorporating ionic pumps and concentration changes. Philos Trans R Soc Lond B Biol Sci 307 (1133):353–98

DiFrancesco P, Tortora D (1991) Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. Nature 351:145–147

DiFrancesco D, Ferroni A, Mazzanti M, Tromba C (1986) Properties of the hyperpolarizing-activated current (i_f) in cells isolated from the rabbit sino-atrial node. J Physiol (London) 377:61–88

Fabiato A, Fabiato F (1979) Calcium programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J Physiol (London) 75:463–505

Ishii TM, Takano M, Xie LH, Noma A, Ohmori H (1999) Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node. J Biol Chem 274:12835–12839

Kaupp UB, Seifert R (2001) Molecular diversity of pacemaker channels. Annu Rev Physiol 63:235–257.

Leoni AL, Marionneau C, Demolombe S, Le Bouter S, Mangoni ME, Escande D, Charpentier F (2005) Chronic heart rate reduction remodels ion channel transcripts in the mouse sinoatrial node but not in the ventricle. Physiol Genomics 24:4–12

Macri V, Proenza C, Agranovich E, Angoli D, Accili EA (2002) Separable gating mechanisms in a mammalian pacemaker channel. J Biol Chem 277:35939–35946

Monnet X, Ghaleb B, Colin P, de Curzon OP, Guidicelli JF, Berdeaux A (2001) Effects of heart rate reduction with Ivabradine on exercise-induced myocardial ischemia and stunning. J Pharmacol Exp Ther 299:1133–1139

Monnet X, Colin P, Ghaleb B, Hittinger L, Giudicelli JF, Berdeaux A (2004) Heart rate reduction during exercise-induced myocardial ischemia and stunning. Eur Heart J 25:579–586

Moreno AP (2004) Biophysical properties of homomeric and heteromultimeric channels formed by cardiac connexins. Cardiovasc Res 62:276–286

Mulder P, Barbier S, Chagraoui A, Richard V, Henry JP, Lallemand F, Renet S, Lerebours G, Mahlberg-Gaudin F, Thuillez C (2004) Long-term heart rate reduction induced by the selective if current inhibitor Ivabradine improves left ventricular function and intrinsic myocardial structure in congestive heart failure. Circulation 109:1674–1679

Rigg L, Mattick PAD, Heath BM, Terrar DA (2003) Modulation of the hyperpolarization-activated current (I_f) by calcium and calmodulin in the guinea-pig sino-atrial node. Cardiovasc Res 57:497–504

Robinson RB, Siegelbaum SA (2003) Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 65:453–480

Rocchetti M, Armato A, Cavalieri B, Micheletti M, Zaza A (1999) Lidocaine inhibition of the hyperpolarization-activated current in sinoatrial myocytes. J Cardiovasc Pharmacol 34:434–439

Romanelli MN, Cerbai E, Dei S, Guandalini L, Martelli C, Martini E, Scapecchi S, Teodori E, Mugelli A (2005) Design, synthesis and preliminary biological evaluation of zatebradine analogues as potential blockers of hyperpolarization-activated current. Bioorg Med Chem 13:1211–1220

Schipke JD, Büter I, Hohlfeld T, Schmitz-Spanke-S, Gams E (2006) Selektive I_f-Kanal-Hemmung: eine Alternative in der Behandlung der koronaren Herzkrankheit? Herz 31:55–74

Simon L, Ghaleb B, Puybasset L, Giudicelli JF, Berdeaux A (1995) Coronary and hemodynamic effects of S 16257, a new bradycardic agent, in resting and exercising conscious dogs. J Pharmacol Exp Ther 275:659–665

Thollon C, Cambarrat C, Vian J, Prost JF, Peglion JL, Vilaine JP (1994) Electrophysiological effects of S 16257, a novel sino-atrial node modulator, on rabbit and guinea-pig cardiac preparations: comparison with UL-FS 49. Br J Pharmacol 112:37–42

Thollon C, Bidouard JP, Cambarrat C, Lesage L, Reure H, Delescluse I, Vian J, Peglion JL, Vilaine JP (1997) Stereospecific in vitro and in vivo effects of the new sinus node inhibitor (+)-S 16257. Eur J Pharmacol 339:43–51

Tsien RW, Rink J (1980) Neutral carrier ion-selective microelectrodes for measurements of intracellular free calcium. Biochim Biophys Acta 599:623–638

Vilaine JP (2004) Selection et caracterisation pharmacologique de Procorolan, un inhibiteur selectif du courant pacemaker If. Therapie 59:495–505

Vilaine JP, Bidouard JP, Lesage L, Reure H, Péglion JL (2003) Anti-ischemic effects of Ivabradine, a selective heart rate-reducing agent, in exercise-induced myocardial ischemia in pigs. J Cardiovasc Pharmacol 42:688–696

Measurement of Cytosolic Calcium with Fluorescent Indicators

Purpose and Rationale

Intracellular free Ca concentration can be measured in cultured endothelial cells with a fluorometric method (Tsien et al. 1982; Grynkiewicz et al. 1985; Lückhoff et al. 1988; Busse and Lamontagne 1991; Hock et al. 1991).

Procedure

Cultured endothelial cells from the pig are seeded on quartz coverslips and grown to confluence. The cells are loaded with the fluorescent probe indo-1 by incubation with 2 μmol indo-1/AM and 0.025 % Pluronic F-127, a nonionic detergent. Thereafter, the coverslips are washed and transferred to cuvettes, filled with HEPES buffer.

Evaluation

Fluorescence is recorded in a temperature controlled (37 °C) spectrofluorophotometer (excitating wavelength 350 nm, emission wavelength simultaneously measured at 400 nm and 450 nm).

Modifications of the Method

Lee et al. (1987) measured cytosolic calcium transients from the beating rabbit heart using indo-1 AM as indicator.

Yanagisawa et al. (1989) measured intracellular Ca^{2+} concentrations in coronary arterial smooth muscle of dogs with fura-2.

Makujina et al. (1995) measured intracellular calcium by fura-2 fluorescence simultaneously with tension in everted rings of porcine coronary artery denuded of endothelium.

Hayashi and Miyata (1994) described the properties of the commonly used fluorescent indicators for intracellular calcium: fura-2, indo-1, and fluo-3.

Monteith et al. (1994) studied the Ca^{2+} pump-mediated efflux in vascular smooth muscles in spontaneously hypertensive rats.

References and Further Reading

Busse R, Lamontagne D (1991) Endothelium-derived bradykinin is responsible for the increase in calcium produced by angiotensin-converting enzyme inhibitors in human endothelial cells. Naunyn Schmiedebergs Arch Pharmacol 344:26–129

Grynkiewicz G, Poenie M, Tsien RY (1985) A new generation of Ca^{+2} indicators with improved fluorescence properties. J Biol Chem 260:3440–3450

Hayashi H, Miyata H (1994) Fluorescence imaging of intracellular Ca^{2+}. J Pharmacol Toxicol Methods 31:1–10

Hock FJ, Wirth K, Albus U, Linz W, Gerhards HJ, Wiemer G, Henke S, Breipohl G, König W, Knolle J, Schölkens BA (1991) Hoe 140 a new potent and long acting bradykinin antagonist: in vitro studies. Br J Pharmacol 102:769–773

Lee HC, Smith N, Mohabir R, Clusin WT (1987) Cytosolic calcium transients from the beating mammalian heart. Proc Natl Acad Sci U S A 84:7793–7797

Lückhoff A, Pohl U, Mülsch A, Busse R (1988) Differential role of extra- and intracellular calcium in the release of EDRF and prostacyclin from cultured endothelial cells. Br J Pharmacol 95:189–196

Makujina SR, Abebe W, Ali S, Mustafa SJ (1995) Simultaneous measurement of intracellular calcium and tension in vascular smooth muscle: validation of the everted ring preparation. J Pharmacol Toxicol Methods 34:157–163

Monteith GR, Chen S, Roufogalis BD (1994) Measurement of Ca^{2+} pump-mediated efflux in hypertension. J Pharmacol Toxicol Methods 31:117–124

Tsien RY, Pozzan T, Rink TJ (1982) Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new intracellularly trapped fluorescent indicator. J Cell Biol 94:325–334

Wiemer G, Popp R, Schölkens BA, Gögelein H (1994) Enhancement of cytosolic calcium, prostaglandin and nitric oxide by bradykinin and the ACE inhibitor ramiprilat in porcine brain capillary endothelial cells. Brain Res 638:261–266

Yanagisawa T, Kawada M, Taira N (1989) Nitroglycerine relaxes canine coronary arterial smooth muscle without reducing intracellular Ca^{2+} concentrations measured with fura-2. Br J Pharmacol 98:469–482

References and Further Reading

Patch-Clamp Technique

Asmild M, Oswald N, Krzywkowski FM, Friis S, Jacobsen RB, Reuter D, Taboryski R, Kutchinsky J, Vestergaard RK, Schroder RL, Sorensen CB, Bech M, Korsgaard MP, Willumsen NJ (2003) Upscaling and automation of electrophysiology: toward high throughput screening in ion channel drug discovery. Receptor Channels 9:49–58

Bennett PB, Guthrie HRE (2003) Trends in ion channel drug discovery: advances in screening technologies. Trends Biotechnol 21:563–569

Brueggemann A, George M, Klau M, Beckler M, Steimdl J, Behrends JC, Fertig N (2004) Ion channel drug discovery and research. The automated nanopatch-clamp technology. Curr Drug Discov Technol 1:91–96

Brüggemann A, Stoelzle S, George M, Behrends JC, Fertig N (2006) Microchip technology for automated and parallel patch-clamp recording. Small 2:840–846

Cavalié A, Grantyn R, Lux HD (1993) Fabrication of patch clamp pipettes. In: Kettenmann H, Grantyn R (eds) Practical electrophysiological methods. Wiley, New York, pp 235–240

Dietzel ID, Bruns D, Polder HR, Lux HD (1993) Voltage clamp recording. In: Kettenmann H, Grantyn R (eds) Practical electrophysiological methods. Wiley, New York, pp 256–262

Falconer M, Smith F, Surah-Narwal S, Congrave G, Liu Z, Hayter P, Ciaramella G, Keighley W, Haddock P, Waldron G, Sewing A (2002) High-throughput screening for ion channel modulators. J Biomol Screen 7:460–465

Fertig N, Klau M, George M, Blick RH, Behrends JC (2002) Activity of single ion channel proteins detected with a planar microstructure. Appl Phys Lett 81:4865–4867

Hamill OP (1993) Cell-free patch clamp. In: Kettenmann H, Grantyn R (eds) Practical electrophysiological methods. Wiley, New York, pp 284–288

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85–100

Jackson MB (1993) Cell-attached patch. In: Kettenmann H, Grantyn R (eds) Practical electrophysiological methods. Wiley, New York, pp 279–283

Krzywkowski K, Schroder RL, Ljungstrom T, Kutchinsky J, Friis S, Vestergaard RK, Jacobsen RB, Pedersen S, Helix N, Sorensen CB, Bech M, Willumsen NJ (2004) Automation of the patch-clamp technique: technical validation through identification and characterization of potassium channel blockers. Biophys J 86:483a

Kutchinsky J, Friis S, Asnild M, Taboryski R, Pedersen S, Vestergaard RK, Jacobsen RB, Krzywkowski K, Schroder RL, Ljungstrom T, Helix N, Sorensen CB, Bech M, Willumsen NJ (2003) Characterization of potassium channel modulators with QPatch automated patch-clamp technology system characteristics and performance. Assay Drug Dev Technol 1:685–693

Liem LK, Simard JM, Song Y, Tewari K (1995) The patch clamp technique. Neurosurgery 36:382–392

Mathes C (2003) Ion channels in drug discovery and development. Drug Discov Today 8:1022–1024

Neher E, Sakmann B (1976) Single-channel currents recorded from membranes of denervated frog muscle fibres. Nature 260:799–802

Sakmann B, Neher E (1995) Single-cell recording. Plenum, New York

Schroeder K, Neagle B, Trezise DJ, Worley J (2003) Ion-Works™ HT: a new high-throughput electrophysiology measurement platform. J Biomol Screen 8:50–64

Smith PA (1995) Methods for studying neurotransmitter transduction mechanisms. J Pharmacol Toxicol Methods 33:63–73

Spencer CI, Li N, Chen Q, Johnson J, Nevill T, Kammonen J, Ionescu-Zanetti C (2012) Ion channel pharmacology under flow: automation via well-plate microfluidics. Assay Drug Dev Technol 10(4):313–324

Patch Clamp Technique in Isolated Cardiac Myocytes

Anno T, Hondeghem LM (1990) Interactions of flecainide with guinea pig cardiac sodium channels. Circ Res 66:789–803

Bennett PB, Stroobandt R, Kesteloot H, Hondeghem LM (1987) Sodium channel block by a potent, new antiarrhythmic agent, transcainide, in guinea pig ventricular myocytes. J Cardiovasc Pharmacol 9:661–667

Bosch RF, Zeng X, Grammer JB, Popovic K, Mewis C, Kühlkamp V (1999) Ionic mechanisms of electrical remodeling in human atrial fibrillation. Cardiovasc Res 44:121–131

Gögelein H, Hartung J, Englert HC, Schölkens BA (1998) HMR 1883, a novel cardioselective inhibitor of the

ATP-sensitive potassium channel. Part I. Effects on cardiomyocytes, coronary flow and pancreatic β-cells. J Pharmacol Exp Ther 286:1453–1464

Gwilt M, Dalrymple HW, Burges RA, Blackburn KJ, Dickinson RP, Cross PE, Higgins AJ (1991) Electrophysiologic properties of UK-66,914, a novel class III antiarrhythmic agent. J Cardiovasc Pharmacol 17:376–385

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85–100

Monyer H, Lambolez B (1995) Molecular biology and physiology at the single-cell level. Curr Opin Neurobiol 5:382–387

Neher E, Sakmann B (1976) Single-channel currents recorded from membranes of denervated frog muscle fibres. Nature 260:799–802

Pallotta BS (1987) Patch-clamp studies of ion channels. In: Meltzer HY (ed) Psychopharmacology: the third generation of progress. Raven, New York, pp 325–331

Ryttsén F, Farre C, Brennan C, Weber SG, Nolkrantz K, Jardemark K, Chiu DT, Orwar O (2000) Characterization of single-cell electroporation by using patch-clamp and fluorescence microscopy. Biophys J 79:1993–2001

Terzic A, Jahangir A, Kurachi Y (1994) HOE-234, a second generation K^+ channel opener, antagonizes the ATP-dependent gating of cardiac ATP-sensitive K^+ channels. J Pharmacol Exp Ther 268:818–825

Tsien RW, Lipscombe D, Madison DV, Bley RK, Fox AP (1988) Multiple types of neuronal calcium channels and their selective modulation. Trends Neurosci 11:431–438

Yazawa K, Kaibara M, Ohara M, Kameyama M (1990) An improved method for isolating cardiac myocytes useful for patch-clamp studies. Jpn J Physiol 40:157–163

Voltage Clamp Studies on Sodium Channels

Abriel H, Wehrens XHT, Benhorin J, Kerem B, Kass RS (2000) Molecular pharmacology of the sodium channel mutation DI790G linked to the long-QT- syndrome. Circulation 102:921–925

Bezanilla F, Armstrong CM (1977) Inactivation of the sodium channel: I. Sodium current experiments. J Gen Physiol 70:549–566

Busch AE, Suessbrich H, Kunzelmann K, Hipper A, Greger R, Waldegger S, Mutschler E, Lindemann B, Lang F (1995) Blockade of epithelial Na^+ channels by triamterenes – underlying mechanisms and molecular basis. Pflugers Arch 432:760–766

Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC (1994) Amiloride-sensitive N^+ channel is made of three homologous subunits. Nature 367:463–467

Catterall WA (1986) Molecular properties of voltage-sensitive sodium channels. Annu Rev Biochem 55:953–985

Eller P, Berjukov S, Wanner S, Huber I, Hering S, Knaus HG, Toth G, Kimball SD, Striessnig J (2000) High affinity interaction of mibefradil with voltage-gated calcium and sodium channels. Br J Pharmacol 130:669–677

Endou H, Hosoyamada M (1995) Potassium-retaining diuretics: aldosterone antagonists. In: Greger RH, Knauf H, Mutschler E (eds) Handbook of experimental pharmacology, vol 117. Springer, Berlin/Heidelberg/New York, pp 335–362

Erdõ SL, Molnár P, Lakics V, Bence JZ, Tömösközi Z (1996) Vincamine and vincanol are potent blockers of voltage-gated Na^+ channels. Eur J Pharmacol 314:69–73

Haeseler G, Leuwer M, Kavan J, Würz A, Dengler R, Piepenbrock S (1999) Voltage-dependent block of normal and mutant muscle sodium channels by 4-Chloro-m-Cresol. Br J Pharmacol 128:1259–1267

Khalifa M, Daleau P, Turgeon J (1999) Mechanism of sodium channel block by venlafaxine in guinea pig ventricular myocytes. J Pharmacol Exp Ther 291:280–284

Makielski JC, Ye B, Valdivia CR, Pagel MD, Pu J, Tester DJ, Ackerman MJ (2003) A ubiquitous splice variant and a common polymorphisms affect heterologous expression of recombinant human SCN5AS heart sodium channels. Circ Res 93:821–828

Nawada T, Tanaka Y, Hisatome I, Sasaki N, Ohtahara A, Kotake H, Mashiba H, Sato R (1995) Mechanism of inhibition of the sodium current by bepridil in guinea-pig isolated ventricular cells. Br J Pharmacol 116:1775–1780

Palmer LG (1992) Epithelial Na channels: function and diversity. Annu Rev Physiol 54:51–66

Ragsdal DS, Numann R, Catterall WA, Scheuer T (1993) Inhibition of Na^+ channels by the novel blocker PD85,639. Mol Pharmacol 43:949–954

Song J-H, Jang Y-Y, Shin Y-K, Lee C-S, Chung S (2000) *N*-Ethyl-maleimide modulation of tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels in rat dorsal root neurons. Brain Res 855:267–273

Sunami A, Hiraoka M (1996) Blockade of cardiac Na^+ channels by a charged class I antiarrhythmic agent, bisaramil: possible interaction of the drug with a pre-open closed state. Eur J Pharmacol 312:245–255

Taglialatela M, Ongini E, Brown AM, di Renzo G, Annunziato L (1996) Felbamate inhibits cloned voltage-dependent Na^+ channels from human and rat brain. Eur J Pharmacol 316:373–377

Viswanathan PV, Bezzina CR, George AL Jr, Roden DM, Wilde AAM, Balser JR (2001) Gating mechanisms for flecainide action in *SNCN5A*-linked arrhythmia syndromes. Circulation 104:1200–1205

Wang DW, Yazawa K, Makita N, George AL Jr, Bennett PB (1997) Pharmacological targeting of long QT mutant sodium channels. J Clin Invest 99:1714–1720

Voltage Clamp Studies on Potassium Channels

Alexander S, Peters J, Mathie A, MacKenzie G, Smith A (2001) TiPS nomenclature supplement 2001

Anson BD, Ackerman MJ, Tester DJ, Will ML, Delisle BP, Anderson CL, January CT (2004) Molecular and functional characterization of common polymorphism in HERG (KCNH2) potassium channels. Am J Physiol 286:H2434–H2441

Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G (1996) KvLQT1 and IsK (minK) proteins associate to form the IKs cardiac potassium current. Nature 384:78–80

Bosch RF, Gaspo R, Busch AE, Lang HJ, Li R-G, Nattel S (1998) Effects of the chromanol 293B, a selective blocker of the slow component of the delayed rectifier K^+ current, on repolarization in human and guinea pig ventricular myocytes. Cardiovasc Res 38:441–450

Busch AE, Suessbrich H, Waldegger S, Sailer E, Greger R, Lang HJ, Lang F, Gibson KJ, Maylie JG (1996) Inhibition of I_{Ks} in guinea pig cardiac myocytes and guinea pig I_{sK} channels by the chromanol 293B. Pflugers Arch 432:1094–1096

Cao Y-J, Dreixler JC, Roizen JD, Roberts MT, Houamed KM (2001) Modulation of recombinant small-conductance Ca^{2+} -activated K^+ channels by the muscle relaxant chlorzoxazone and structurally related compounds. J Pharmacol Exp Ther 296:683–689

Carmeliet A, Mubagawa K (1998) Antiarrhythmic drugs and cardiac ion channels: mechanism of action. Prog Biophys Mol Biol 70:1–71

Chabbert C, Chambard JM, Sans A, Desmadryl G (2001) Three types of depolarization-activated potassium currents in acutely isolated mouse vestibular neurons. J Neurophysiol 85:1017–1026

Champeroux P, Guth BD, Markert M, Rast G (2013) Methods in Cardiovascular Safety Pharmacology. In: Vogel HG, Maas J, Hock FJ, Mayer D (eds) Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays, 2nd edn. Springer Berlin Heidelberg. p. 73–97

Colatsky TJ, Follmer CH, Starmer CF (1990) Channel specificity in antiarrhythmic drug action. Mechanism of potassium channel block and its role in suppressing and aggravating cardiac arrhythmias. Circulation 82:2235–2242

Escande D, Henry P (1993) Potassium channels as pharmacological targets in cardiovascular medicine. Eur Heart J 14(Suppl B):2–9

Gintant GA (1996) Two components of delayed rectifier current in canine atrium and ventricle. Does I_{Ks} play a role in the reverse rate dependence of class III agents? Circ Res 78:26–37

Gögelein H, Brüggemann A, Gerlach U, Brendel J, Busch AE (2000) Inhibition of I_{Ks} channels by HMR 1556. Naunyn-Schmiedebergs Arch Pharmacol 362:480–488

Golding AL (1992) Maintenance of *Xenopus laevis* and oocyte injection. Methods Enzymol 207:266–279

Grissmer S, Nguyen AN, Aiyar J, Hanson DC, Mather RJ, Gutman GA, Karmilowicz MJ, Auperin DD, Chandy KG (1994) Pharmacological characterization of five cloned voltage-gated K^+ channels, types Kv1.1, 1.2, 1.3, 1.5, and 3.1, stably expressed in mammalian cell lines. Mol Pharmacol 45:1227–1234

Kaczorowski GJ, Garcia ML (1999) Pharmacology of voltage-gated and calcium-activates potassium channels. Curr Opin Chem Biol 3:448–458

Lei M, Brown HF (1996) Two components of the delayed rectifier potassium current, IK, in rabbit sinoatrial node cells. Exp Physiol 81:725–741

Longobardo M, Delpón E, Caballero R, Tamargo J, Valenzuela C (1998) Structural determinants of potency and stereoselective block of hKv1.5 channels induced by local anesthetics. Mol Pharmacol 54:162–169

Methfessel C, Witzemann V, Takahashi T, Mishina M, Numa S, Sakmann B (1986) Patch clamp measurements on *Xenopus laevis* oocytes: currents through endogenous channels and implanted acetylcholine receptor. Pflugers Arch 407:577–588

Moreno I, Caballero R, Ganzález T, Arias C, Valenzuela C, Iriepa I, Gálvez E, Tamargo J, Delpón E (2003) Effects of irbesartan on cloned potassium channels involved in human cardiac repolarization. J Pharmacol Exp Ther 304:862–873

Sanchez-Chapula JA (1999) Mechanisms of transient outward K^+ channel block by disopyramide. J Pharmacol Exp Ther 290:515–523

Sanchez-Chapula JA, Navarro-Polanco RA, Culberson C, Chen J, Sanguinetti MC (2002) Molecular determinants of voltage-dependent human ether-a-go-go related gene (HERG) K^+ channel block. J Biol Chem 277:23587–23595

Sanguinetti MC, Jurkiewicz NK (1990) Two components of cardiac delayed rectifier K^+ currents: differential sensitivity to block by class III antiarrhythmic agents. J Gen Physiol 96:195–215

Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MD (1996) Coassembly of KvLTQ1 and minK (IsK) proteins form cardiac IKs potassium channel. Nature 384:80–83

Suessbrich H, Busch A, Ecke D, Rizzo M, Waldegger S, Lang F, Szabo I, Lang HJ, Kunzelmann K, Greger R, Busch AE (1996) Specific blockade of slowly activating channels by chromanols – impact on the role of I_{sK} channels in epithelia. FEBS Lett 396:271–275

Suessbrich H, Busch AE, Scherz MW (1997) The pharmacology of cloned cardiac potassium channels. Ion Channel Modulators 2:432–439

Tagliatela M, Timmerman H, Annunziato L (2000) Cardiotoxic potential and CNS effects of first-generation antihistamines. Trends Pharmacol Sci 21:52–65

Vandenberg JI, Walker BD, Campbell TC (2001) HERG K^+ channels: friend and foe. Trends Pharmacol Sci 22:240–246

Wang Z, Fermini B, Nattel S (1994) Rapid and slow components of delayed rectifier current in human atrial myocytes. Cardiovasc Res 28:1540–1546

Zhou J, Angelli-Szafran CE, Bradley JA, Chen X, Koci BJ, Volberg WA, Sun Z, Cordes JS (2005) Novel potent human *Ether-à-Go-Go*-related gene (*h*ERG) potassium channel enhancers and their in vitro antiarrhythmic activity. Mol Pharmacol 68:876–884

Studies on Kv1.5 Channel

Bachmann A, Gutcher I, Kopp K, Brendel J, Bosch RF, Busch AE, Gögelein H (2001) Characterization of a novel Kv1.5 channel blocker in Xenopus oocytes, CHO cells, and human cardiomyocytes. Naunyn-Schmiedebergs Arch Pharmacol 364:472–478

Caballero R, Delpón E, Valenzuela C, Longobardo M, Tamargo J (2000) Losartan and its metabolite E3174 modify cardiac delayed rectifier K^+ currents. Circulation 101:1199–1205

Caballero R, Delpón E, Valenzuela C, Longobardo M, González T, Tamagro J (2001) Direct effects of candesartan and eprosartan in human cloned potassium channels involved in cardiac repolarization. Mol Pharmacol 59:825–836

Caballero R, Gómez R, Núòez L, Moreno I, Tamargo J, Delpón E (2004) Diltiazem inhibits hKv1.5 and Kv4.3 currents in therapeutic concentrations. Cardiovasc Res 64:457–466

Choe H, Lee YK, Lee YT, Choe H, Ko SH, Joo CU, Kim MH, Kim GS, Eun JS, Kim JH, Chae SW, Kwak YG (2003) Papaverine blocks hKv1.5 channel current and human atrial ultrarapid delayed rectifier K^+ currents. J Pharmacol Exp Ther 304:706–712

Choi BH, Choi JS, Rhie DJ, Yoon SH, Min DS, Jo YH, Kim MS, Hahn SJ (2002) Direct inhibition of the cloned Kv1.5 channel by AG-1478, a tyrosine kinase inhibitor. Am J Physiol 282:C1461–C1468

Decher N, Uyguner O, Scherer CR, Karaman B, Yüksel-Apak M, Busch AE, Steinmeyer K, Wollnik B (2001) hKChIP2 is a functional modifier of hKv4.3 potassium channels: cloning and expression of a short hKChIP2 splice variant. Cardiovasc Res 52:255–264

Dilks D, Ling H-P, Cockett M, Sokol P, Numann R (1999) Cloning and expression of the human Kv4.3 potassium channel. J Neurophysiol 81:1974–1977

Fedida D, Eldstrom J, Hesketh C, Lamorgese M, Castel L, Steele DF, van Wagoner DR (2003) Kv1.5 is an important component of repolarizing K^+ current in canine atrial myocytes. Circ Res 93:744–751

Godreau D, Vranckx R, Hatem SN (2002) Mechanism of action of antiarrhythmic agent bertosamil on hKv1.5 channels and outward current in human atrial myocytes. J Pharmacol Exp Ther 300:612–620

Godreau D, Vranckx R, Maguy A, Goyenvalle C, Hatem SN (2003) Different isoforms of synapse-associated protein, SAP97, are expressed in the heart and have distinct effects on the voltage-gated K^+ channel Kv1.5. J Biol Chem 278:47046–47052

Gögelein H, Hartung J, Englert HC, Schölkens BA (1998) HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. I. Effects on cardiomyocytes, coronary flow and pancreatic β-cells. J Pharmacol Exp Ther 286:1453–1464

Gögelein H, Brendel J, Steinmeyer K, Strübing C, Picard N, Rampe D, Kopp K, Busch AE, Bleich M (2004) Effects of the antiarrhythmic drug AVE0118 on cardiac ion channels. Naunyn-Schmiedebergs Arch Pharmacol 370:183–192

Hamill OP, Marty M, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85–100

Kobayashi S, Reien Y, Ogura T, Saito T, Masuda Y, Nakaya H (2001) Inhibitory effect of bepridil on hKv1.5 channel current; comparison with amiodarone and E-4031. Eur J Pharmacol 430:149–157

Krause E, Englert H, Gögelein H (1995) Adenosine triphosphate-dependent K currents activated by metabolic inhibition in rat ventricular myocytes differ from those elicited by the channel opener rilmakalim. Pflugers Arch 429:625–635

Li GR, Feng J, Wang Z, Fermine B, Nattel S (1996) Adrenergic modulation of ultrarapid rectifier K^+ current in human atrial myocytes. Circ Res 78:903–915

Longobardo M, González T, Navarro-Polanco R, Calballero R, Delpón E, Tamargo J, Snyders DJ, Tamkum MM, Valenzuela C (2000) Effects of a quaternary bupivacaine derivative on delayed rectifier K^+ currents. Br J Pharmacol 130:391–401

Matsuda T, Masumiya H, Tanaka N, Yamashita T, Tsuruzoe N, Tanaka Y, Tanaka H, Shigenoba K (2001) Inhibition by a novel anti-arrhythmic agent, NIP-142, of cloned human cardiac K^+ channel Kv1.5 current. Life Sci 68:2017–2024

Moreno I, Caballero R, González T, Arias C, Valenzuela C, Iriepa I, Gálvez E, Tamargo J, Delpón E (2003) Effects of irbesartan on cloned potassium channels involved in human cardiac repolarization. J Pharmacol Exp Ther 304:862–873

Perchenet L, Clément-Chomienne O (2000) Characterization of the mibefradil block of the human heart delayed rectifier hKv1.5. J Pharmacol Exp Ther 295:771–778

Peukert S, Brendel J, Pirad B, Bruggemann A, Below P, Kleemann HW, Hemmerle H, Schmidt W (2003) Identification, synthesis, and activity of novel blockers of the voltage-gated potassium channel Kv1.5. J Med Chem 46:486–498

Peukert S, Brendel J, Pirard B, Strübing C, Kleemann HW, Böhme T, Hemmerle H (2004) Pharmacophore-based search, synthesis, and biological evaluation of anthranilic amides as novel blockers of the Kv1.5 channel. Bioorg Med Chem Lett 14:2823–2827

Plane F, Johnson R, Kerr P, Wiehler W, Thorneloe K, Ishii K, Chen T, Cole W (2005) Heteromultimeric

Kv1 channels contribute to myogenic control of arterial diameter. Circ Res 96:216–224
Rampe D, Roy ML, Dennis A, Brown AM (1997) A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. FEBS Lett 417:28–32
Villmann C, Bull L, Hollmann M (1997) Kainate binding proteins possess functional ion channel domains. J Neurosci 17:7634–7643
Wirth KJ, Knobloch K (2001) Differential effects of dofetilide, amiodarone, and class Ic drugs on left and right atrial refractoriness and left atrial vulnerability in pigs. Naunyn-Schmiedebergs Arch Pharmacol 363:166–174

Voltage Clamp Studies on Calcium Channels

Augustine GJ, Charlton MP, Smith RJ (1987) Calcium action in synaptic transmitter release. Annu Rev Neurosci 10:633–693
Bean BP (1989) Classes of calcium channels in vertebrate cells. Annu Rev Physiol 51:376–384
Berjukom S, Marksteiner R, Gapp F, Sinneger MJ, Hering S (2000) Molecular mechanism of calcium channel block by isradipine. Role of a drug-induced inactivated channel configuration. J Biol Chem 275:22114–22120
Catterall WA (2000) Structure and regulation of voltage-gated Ca^{2+} channels. Ann Rev Cell Dev Biol 16:521–555
Cheng T-H, Lee F-Y, Wei J, Lin C-I (1996) Comparison of calcium-current in isolated atrial myocytes from failing and nonfailing human hearts. Mol Cell Biochem 157:157–162
Foehring RC, Srcoggs RS (1994) Multiple high-threshold calcium currents in acutely isolated rat amygdaloid pyramidal cells. J Neurophysiol 71:433–436
Gomez JP, Potreau D, Branka JE, Raymond G (1994) Developmental changes in Ca^{2+} currents from newborn rat cardiomyocytes in primary culture. Pflugers Arch 428:214–249
Hering S, Aczél S, Klaus RL, Berjukow S, Striessnig J, Timin EN (1997) Molecular mechanism of use-dependent calcium channel block by phenylalkylamines: role of inactivation. Proc Natl Acad Sci U S A 94:13323–13328
Hockerman GH, Peterson BZ, Sharp E, Tanada TN, Scheuer T, Catterall WA (1997) Constriction of a high-affinity receptor site for dihydropyridine agonists and antagonists by single amino acid substitution in a non-L-type Ca^{2+} channel. Proc Natl Acad Sci USA 94:14906–14911
Johnson BD, Scheuer T, Catterall WA (1994) Voltage-dependent potentiation of L-type Ca^{2+} channels in skeletal muscles cells requires anchored cAMP-dependent protein kinase. Proc Natl Acad Sci 91:11492–11496
Koester HJ, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagation action potentials and subthreshold excitatory potentials. Proc Natl Acad Sci U S A 95:9596–9601
Kraus RL, Hering S, Grabner M, Ostler D, Striessnig J (1998) Molecular mechanisms of diltiazem interaction with L-type Ca^{2+} channels. J Biol Chem 273:27205–27212
Lacinová L, An HR, Xia J, Ito H, Klugbauer N, Triggle E, Hofmann F, Kass RS (1999) Distinction in the molecular determinants of charged and neutral dihydropyridine block of L-type calcium channels. J Pharmacol Exp Ther 289:1472–1479
Lacinová L, Klugbauer N, Hofmann F (2000) State- and isoform-dependent interaction of isradipine with the α_{1C} L-type calcium channel. Pflügers Arch Eur J Physiol 440:50–60
Margrie TW, Sakmann B, Urban NN (2001) Action potential propagation in mitral cell lateral dendrites is decremental and controls recurrent and lateral inhibition in the mammalian olfactory bulb. Proc Natl Acad Sci U S A 98:319–324
Markram H, Sakmann B (1994) Calcium transients in dendrites of neocortical neurons evoked by single subthreshold excitatory postsynaptic potentials via low-voltage-activated calcium channels. Proc Natl Acad Sci U S A 91:5207–5211
McHugh D, Sharp EM, Scheuer T, Catterall WA (2000) Inhibition of L-type calcium channels by protein kinase C phosphorylation of two sites in the N-terminal domain. Proc Natl Acad Sci U S A 97:12334–12338
Miller RJ (1987) Multiple calcium channels and neuronal function. Science 235:46–52
Morita H, Cousins H, Inoue H, Ito Y, Inoue R (1999) Predominant distribution of nifedipine-insensitive, high voltage-activated Ca^{2+} channels in the terminal mesenteric artery of guinea pig. Circ Res 85:596–605
Narahash T, Tsunoo A, Yoshii M (1987) Characterization of two types of calcium channels in mouse neuroblastoma cells. J Physiol 38:231–249
Peterson BZ, Johnson BD, Hockerman GH, Acheson M, Scheuer T, Catterall WA (1997) Analysis of the dihydropyridine receptor site of L-type calcium channels by alanine-scanning mutagenesis. J Biol Chem 272:18752–18758
Scamps F, Mayoux E, Charlemagne D, Vassort G (1990) Calcium current in single cells isolated from normal and hypertrophied rat heart. Circ Res 67:199–208
Sculptoreanu A, Rotman E, Takahasi M, Scheuer T, Catterall WA (1993) Voltage-dependent potentiation of the activity of cardiac L-type calcium channel α1 subunits due to phosphorylation by cAMP-dependent protein kinase. Proc Natl Acad Sci U S A 90:10135–10139
Snutch TP, Sutton KG, Zamponi GW (2001) Voltage-dependent calcium channels – beyond dihydropyridine antagonists. Curr Opin Pharmacol 1:11–16

Stephens GJ, Page KM, Burley JR, Berrow NS, Dolphin AC (1997) Functional expression of brain cloned α1E calcium channels in COS-7 cells. Pflugers Arch 433:525–532

Stuart G, Spruston N (1995) Probing dendritic function with patch pipettes. Curr Opin Neurobiol 5:389–394

Tohse N, Masuda H, Sperelakis N (1992) Novel isoform of Ca^{2+} channel in rat fetal cardiomyocytes. J Physiol (London) 451:295–306

Waard MD, Campell KP (1995) Subunit regulation of the neuronal α_{1A} Ca^{2+} channel expressed in *Xenopus* oocytes. J Physiol (London) 485:619–634

Wu S, Zhang M, Vest PA, Bhattacharjee A, Liu L, Li M (2000) A mifrabidile metabolite is a potent intracellular blocker of L-type Ca^{2+} currents in pancreatic β-cells. J Pharmacol Exp Ther 292:939–943

Xu X, Rials SJ, Wu Y, Liu T, Marinchak RA, Kowey PR (2000) Effects of captopril treatment of renovascular hypertension on β-adrenergic modulation of L-type Ca^{2+} current. J Pharmacol Exp Ther 292:196–200

Yang JC, Shan J, Ng KF, Pang P (2000) Morphine and methadone have different effects on calcium channel currents in neuroblastoma cells. Brain Res 870:199–203

Young C, Huang Y-C, Lin C-H, Shen Y-Z, Gean P-W (2001) Selective enhancement of L-type calcium currents by corticotropin in acutely isolated rat amygdala neurons. Mol Pharmacol 59:604–611

Zamponi GW (1997) Antagonist sites of voltage-dependent calcium channels. Drug Dev Res 42:131–143

Patch Clamp Studies on Chloride Channels

Cliff WH, Frizzell RA (1990) Separate Cl- conductances activated by cAMP and Ca^{2+} in Cl(-)-secreting epithelial cells. Proc Natl Acad Sci U S A 87(13):4956–60

Frings S, Reuter D, Kleene SJ (2000) Neuronal Ca^{2+} activated Cl^- channels – homing in on an elusive channel species. Prog Neurobiol 60:247–289

Jentsch TJ, Günther W (1997) Chloride channels: an emerging molecular picture. Bioessays 19:117–126

Maertens C, Wie L, Droogmans G, Nilius B (2000) Inhibition of volume-regulated and calcium-activated chloride channels by the antimalarial mefloquine. J Pharmacol Exp Ther 295:29–36

Pusch M, Liantonio A, Bertorello L, Accardi A, de Lucca A, Pierno S, Tortorella V, Camerino DC (2000) Pharmacological characterization of chloride channels belonging to the ClC family by the use of chiral clofibric acid derivatives. Mol Pharmacol 58:498–507

Inhibition of Hyperpolarization-Activated Channels

Albaladejo P, Challande P, Kakou A, Benetos A, Labat C, Louis H, Safar ME, Lacolley P (2004) Selective reduction of heart rate by ivabradine: effect on the viscoelastic arterial properties in rats. J Hypertens 22:1739–1745

Accili EA, DiFrancesco D (1996) Inhibition of the hyperpolarization-activated current (if) of rabbit SA node myocytes by niflumic acid. Pflugers Arch 431:757–762

Accili EA, Robinson RB, DiFrancesco D (1997) Properties and modulation of I_f in newborn versus adult SA node. Am J Physiol 272:H1549–H1552

Accili EA, Proenza C, Baruscotti M, DiFrancesco D (2002) From funny current to HCN channels: 20 years of excitation. News Physiol Sci 17:32–37

Baruscotti M, Bucchi A, DiFrancesco D (2005) Physiology and pharmacology of the cardiac pacemaker ("funny") current. Pharmacol Ther 107:59–79

Biel M, Schneider A, Wahl C (2002) Cardiac HCN channels: structure, function, and modulation. Trends Cardiovasc Med 12:206–2134

Bois P, Bescond J, Renaudon B, Lenfant J (1996) Mode of action of bradycardic agent, S-16257, on ionic currents of rabbit sinoatrial node cells. Br J Pharmacol 118:1051–1057

Bucchi A, Baruscotti M, DiFrancesco D (2002) Current-dependent block of rabbit sino-atrial node I_f channels by Ivabradine. J Gen Physiol 120:1–13

Cerbai E, de Paoli P, Sartiani L, Lonardo G, Mugelli A (2003) Treatment with Irbesartan counteracts the functional remodeling of ventricular myocytes from hypertensive rats. J Cardiovasc Pharmacol 41:804–812

Chatelier A, Renaudon B, Bescond J, El Chemaly A, Demion M, Bois P (2005) Calmodulin antagonist W7 directly inhibits f-type current in rabbit sino-atrial cells. Eur J Pharmacol 521:29–33

Colin P, Ghaleb B, Monnet X, Hittinger L, Berdeaux A (2004) Effect of graded heart rate reduction with Ivabradine on myocardial oxygen consumption and diastolic time in exercising dogs. J Pharmacol Exp Ther 308:236–240

Deplon E, Valenzuela C, Perez O, Franqueza L, Gay P, Snyders DJ, Tamargo J (1996) Mechanisms of block of human cloned potassium channel by the enantiomers of a new bradycardic agent: S-16257–2 and S-1620–2. Br J Pharmacol 117:1293–1301

DiFrancesco D, Camm JA (2004) Heart rate lowering by specific and selective I_f current inhibition with Ivabradine. Drugs 64:1757–1765

DiFrancesco D, Mangoni M (1994) Modulation of single hyperpolarization-activated channels i_f by cAMP in the rabbit sino-atrial node. J Physiol (London) 474:473–482

DiFrancesco D, Noble D (1985) A model of cardiac electrical activity incorporating ionic pumps and concentration changes. Philos Trans R Soc Lond B Biol Sci 307(1133):353–98

DiFrancesco P, Tortora D (1991) Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. Nature 351:145–147

DiFrancesco D, Ferroni A, Mazzanti M, Tromba C (1986) Properties of the hyperpolarizing-activated current (i_f) in cells isolated from the rabbit sino-atrial node. J Physiol (London) 377:61–88

Fabiato A, Fabiato F (1979) Calcium programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J Physiol (London) 75:463–505

Ishii TM, Takano M, Xie LH, Noma A, Ohmori H (1999) Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node. J Biol Chem 274:12835–12839

Kaupp UB, Seifert R (2001) Molecular diversity of pacemaker channels. Annu Rev Physiol 63:235–257

Leoni AL, Marionneau C, Demolombe S, Le Bouter S, Mangoni ME, Escande D, Charpentier F (2005) Chronic heart rate reduction remodels ion channel transcripts in the mouse sinoatrial node but not in the ventricle. Physiol Genomics 24:4–12

Macri V, Proenza C, Agranovich E, Angoli D, Accili EA (2002) Separable gating mechanisms in a mammalian pacemaker channel. J Biol Chem 277:35939–35946

Monnet X, Ghaleb B, Colin P, de Curzon OP, Guidicelli JF, Berdeaux A (2001) Effects of heart rate reduction with Ivabradine on exercise-induced myocardial ischemia and stunning. J Pharmacol Exp Ther 299:1133–1139

Monnet X, Colin P, Ghaleb B, Hittinger L, Giudicelli JF, Berdeaux A (2004) Heart rate reduction during exercise-induced myocardial ischemia and stunning. Eur Heart J 25:579–586

Moreno AP (2004) Biophysical properties of homomeric and heteromultimeric channels formed by cardiac connexins. Cardiovasc Res 62:276–286

Mulder P, Barbier S, Chagraoui A, Richard V, Henry JP, Lallemand F, Renet S, Lerebours G, Mahlberg-Gaudin F, Thuillez C (2004) Long-term heart rate reduction induced by the selective if current inhibitor Ivabradine improves left ventricular function and intrinsic myocardial structure in congestive heart failure. Circulation 109:1674–1679

Rigg L, Mattick PAD, Heath BM, Terrar DA (2003) Modulation of the hyperpolarization-activated current (I_f) by calcium and calmodulin in the guinea-pig sino-atrial node. Cardiovasc Res 57:497–504

Robinson RB, Siegelbaum SA (2003) Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 65:453–480

Rocchetti M, Armato A, Cavalieri B, Micheletti M, Zaza A (1999) Lidocaine inhibition of the hyperpolarization-activated current in sinoatrial myocytes. J Cardiovasc Pharmacol 34:434–439

Romanelli MN, Cerbai E, Dei S, Guandalini L, Martelli C, Martini E, Scapecchi S, Teodori E, Mugelli A (2005) Design, synthesis and preliminary biological evaluation of zatebradine analogues as potential blockers of hyperpolarization-activated current. Bioorg Med Chem 13:1211–1220

Schipke JD, Büter I, Hohlfeld T, Schmitz-Spanke S, Gams E (2006) Selektive I_f-Kanal-Hemmung: eine Alternative in der Behandlung der koronaren Herzkrankheit? Herz 31:55–74

Simon L, Ghaleb B, Puybasset L, Giudicelli JF, Berdeaux A (1995) Coronary and hemodynamic effects of S 16257, a new bradycardic agent, in resting and exercising conscious dogs. J Pharmacol Exp Ther 275:659–665

Thollon C, Cambarrat C, Vian J, Prost JF, Peglion JL, Vilaine JP (1994) Electrophysiological effects of S 16257, a novel sino-atrial node modulator, on rabbit and guinea-pig cardiac preparations: comparison with UL-FS 49. Br J Pharmacol 112:37–42

Thollon C, Bidouard JP, Cambarrat C, Lesage L, Reure H, Delescluse I, Vian J, Peglion JL, Vilaine JP (1997) Stereospecific in vitro and in vivo effects of the new sinus node inhibitor (+)-S 16257. Eur J Pharmacol 339:43–51

Tsien RW, Rink J (1980) Neutral carrier ion-selective microelectrodes for measurements of intracellular free calcium. Biochim Biophys Acta 599:623–638

Vilaine JP (2004) Selection et caracterisation pharmacologique de Procorolan, un inhibiteur selectif du courant pacemaker If. Therapie 59:495–505

Vilaine JP, Bidouard JP, Lesage L, Reure H, Péglion JL (2003) Anti-ischemic effects of Ivabradine, a selective heart rate-reducing agent, in exercise-induced myocardial ischemia in pigs. J Cardiovasc Pharmacol 42:688–696

Measurement of Cytosolic Calcium with Fluorescent Indicators

Busse R, Lamontagne D (1991) Endothelium-derived bradykinin is responsible for the increase in calcium produced by angiotensin-converting enzyme inhibitors in human endothelial cells. Naunyn Schmiedebergs Arch Pharmacol 344:26–129

Grynkiewicz G, Poenie M, Tsien RY (1985) A new generation of Ca^{+2} indicators with improved fluorescence properties. J Biol Chem 260:3440–3450

Hayashi H, Miyata H (1994) Fluorescence imaging of intracellular Ca^{2+}. J Pharmacol Toxicol Methods 31:1–10

Hock FJ, Wirth K, Albus U, Linz W, Gerhards HJ, Wiemer G, Henke S, Breipohl G, König W, Knolle J, Schölkens BA (1991) Hoe 140 a new potent and long acting bradykinin antagonist: *in vitro* studies. Br J Pharmacol 102:769–773

Lee HC, Smith N, Mohabir R, Clusin WT (1987) Cytosolic calcium transients from the beating mammalian heart. Proc Natl Acad Sci U S A 84:7793–7797

Lückhoff A, Pohl U, Mülsch A, Busse R (1988) Differential role of extra- and intracellular calcium in the release of EDRF and prostacyclin from cultured endothelial cells. Br J Pharmacol 95:189–196

Makujina SR, Abebe W, Ali S, Mustafa SJ (1995) Simultaneous measurement of intracellular calcium and tension in vascular smooth muscle: validation of the everted ring preparation. J Pharmacol Toxicol Methods 34:157–163

Monteith GR, Chen S, Roufogalis BD (1994) Measurement of Ca^{2+} pump-mediated efflux in hypertension. J Pharmacol Toxicol Methods 31:117–124

Tsien RY, Pozzan T, Rink TJ (1982) Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new intracellularly trapped fluorescent indicator. J Cell Biol 94:325–334

Wiemer G, Popp R, Schölkens BA, Gögelein H (1994) Enhancement of cytosolic calcium, prostaglandin and nitric oxide by bradykinin and the ACE inhibitor ramiprilat in porcine brain capillary endothelial cells. Brain Res 638:261–266

Yanagisawa T, Kawada M, Taira N (1989) Nitroglycerine relaxes canine coronary arterial smooth muscle without reducing intracellular Ca^{2+} concentrations measured with fura-2. Br J Pharmacol 98:469–482