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Analysis of the hyperpolarizing effects of forskolin in guinea-pig atrial heart muscle

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Summary. The effects of forskolin on action potential configuration and on both uptake and efflux of $^{86}Rb^+$ were studied in guinea-pig left atria. The action potential was prolonged by forskolin in the plateau range but shortened at the end of repolarization; maximal upstroke velocity and amplitude of slow response potentials were enhanced. In partially depolarized preparations, the resting potential was increased by forskolin; this effect was not prevented by atropine 1 μmol/l. Forskolin augmented the rate constant of $^{86}Rb^+$ efflux in beating and in resting preparations. The uptake of $^{86}Rb^+$ was enhanced by forskolin in resting preparations. It is concluded that forskolin stimulates the Na^+,K^+-pump and activates a background potassium conductance. Both effects may account for the shortening effect of the drug on the action potential and the increase in resting potential seen in partially depolarized preparations.

Key words: Guinea-pig atrium – Forskolin – Action potential – Resting potential – $^{86}Rb^+$ efflux – $^{86}Rb^+$ uptake

Introduction

Forskolin increases the force of contraction of the heart and lowers the blood pressure in various animals (Lindner et al. 1978). Subsequent studies showed that forskolin directly activates cardiac adenylate cyclase and increases the activity of cyclic AMP-dependent protein kinase (Metzger and Lindner 1981). The activation by forskolin of the adenylate cyclase in a number of tissues and an increase in cyclic AMP levels in brain slices was shown by Seamon et al. (1981).

The positive inotropic effect of forskolin in guinea-pig papillary muscles was described as being associated with either a shortening of the action potential (Lindner et al. 1978) or a slight prolongation (Späh 1984), whereas large increases by forskolin in action potential duration were described in single myocytes isolated from guinea-pig ventricles (Rardon and Pappano 1986). Forskolin increases the slow inward calcium current (I_{Ca}) in isolated myocytes (Hescheler et al. 1986), which is in line with the observed prolongation of the action potential. However, other effects of forskolin than the increase in I_{Ca} may determine the overall effects of the drug on the action potential configuration in heart tissue. It was the aim of the present study to characterize the opposing effects of forskolin on the action potential configuration in guinea-pig atrial heart muscle. To this end, first, the effects of forskolin on maximal upstroke velocity, diastolic potential and the duration of the action potential at 20% and 90% of repolarization were determined. Second, the changes by forskolin in maximal upstroke velocity of slow response action potentials were investigated. Third, the effects of forskolin on the maximal diastolic potential were determined in preparations partially depolarized by Ba^{2+} 0.2 mmol/l. To find a logical explanation for the abbreviation by forskolin of the action potential, experiments were carried out with $^{86}Rb^+$ as a probe for both the activity of the Na^+,K^+-pump (uptake measurements) and the potassium conductance (efflux measurements) of the cell membrane. The results of the present study suggest that forskolin, in addition to its effect on I_{Ca}, stimulates the Na^+,K^+-pump and increases a steady state potassium conductance as well. Both effects may contribute to the hyperpolarizing effects of forskolin in guinea-pig atrial heart muscle.

Methods

Preparations. For electrophysiological studies and the measurement of $^{86}Rb^+$ uptake or $^{86}Rb^+$ efflux, guinea-pigs of weight 250–400 g were killed by a blow on the head and bled from the carotid arteries. The hearts were quickly removed and transferred to a dissection chamber containing oxygenated warm Tyrode's solution. Whole hearts were pinned down on Sylgard to cut off the atria. Suitable left atrial trabeculae, mean dimensions ± SD 3.5 ± 1.0 mm length × 0.4 ± 0.1 mm diameter, were selected under 6–10-fold magnification and isolated by ligating both ends with a fine silk suture and dissecting them from the atrium. For flux studies, the whole left atrial appendage was used.

Solutions. Tyrode's solution was prepared from stock solutions in distilled deionized water and had the following composition in mmol/l: NaCl, 136.9; KCl, 5.4; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 11.9; $CaCl_2$, 1.8; glucose, 5.6. The Tyrode's solution was equilibrated with 5% CO_2 in O_2 at 37°C (pH 7.4). High potassium depolarizing modified Tyrode's solution contained 21.6 mmol/l potassium by replacing the NaCl with equimolar amounts of KCl (other ions the same). $BaCl_2$ 0.2 mmol/l was added to the solution containing 21.6 mmol/l potassium.

Measurement of transmembrane potentials of heart muscle preparations. The atrial trabeculae were mounted

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horizontally in a 2 ml organ bath, which was built into a perspex block that also contained a main reservoir of 100 ml Tyrode's solution. Communication between both compartments was provided by connecting pores through which the fluids were driven by gas (95% O_2 and 5% CO_2). The preparations were electrically stimulated at 1 Hz by rectangular pulses of 0.1 – 1 ms duration at 10% above threshold intensity and allowed to stabilize for at least 30 min. The effects of forskolin were investigated by exposure to either single or to cumulatively increasing concentrations, achieved by adding drugs to the main Tyrode's reservoir, and increasing the concentration after the establishment of a stable response. Slow response action potentials were recorded by increasing the extracellular potassium concentration from 5.4 mmol/l to 21.6 mmol/l. Under these conditions (resting potential of about −52 mV), the sodium conductance responsible for the fast upstroke of the action potential is virtually completely inactivated and calcium-dependent slow response action potentials can be recorded (originally described by Reuter and Scholz 1968). $BaCl_2$ 0.2 mmol/l was added to guarantee the homogenous propagation of slow responses (Ehara and Inazawa 1980). For the evaluation of hyperpolarizing drug effects, preparations were partially depolarized by $BaCl_2$ 0.2 mmol/l in normal Tyrode's solution. In these experiments, the driving frequency was set to 2 Hz to overdrive spontaneous activity which was generally induced by forskolin at the lower resting potential. The transmembrane potential was detected intracellularly by the use of 10 – 20 MΩ glass microelectrodes filled with KCl 3 mol/l. The signals were led off by means of a voltage follower with input capacity compensation. The transmembrane potential was displayed on a cathode ray oscilloscope (Tektronix 5103N) and recorded on magnetic tape (Lyrec FM tape recorder, bandwidth 0 – 10 kHz). For the determination of the maximal upstroke velocity, the first time derivative of the action potential was obtained by analogue differentiation and recorded on tape. During the course of an experiment, all parameters could be observed on a digital scope (Nicolet Explorer I). For quantitative evaluation, all data were stored, amplified and edited by means of a transient recorder (Physical Data, 512A) and written out to an XY-pen recorder (MFE 815).

Measurement of $^{86}Rb^+$ efflux. $^{86}Rb^+$ was used in the present study to show the effect of forskolin on the potassium conductance. $^{86}Rb^+$ has a longer half life than $^{42}K^+$, is therefore easier to handle and can be used, within certain limitations, as a tracer substance substituting for potassium. Rb^+ ions presumably pass the potassium channels of atrial heart muscle to a significantly smaller extent than potassium ions. In resting guinea-pig atria, the time constants of the efflux of $^{42}K^+$ amounted to about 50 min (Nawrath et al. 1980) and that of $^{86}Rb^+$ to about 100 min (this paper). The difference points to a reduced permeability of potassium channels for Rb^+ ions and is in line with results obtained in nerve cell membranes (Plant 1986). Further evidence for the assumption that Rb^+ passes through potassium channels in guinea-pig atrial heart muscle is given by the fact that the efflux of $^{86}Rb^+$ is inhibited by $BaCl_2$ (unpublished work).

Whole guinea-pig left atria were first exposed to about 10 MBq $^{86}Rb^+$ (specific activity about 80 GBq/g) for 90 min in Tyrode's solution and then transferred to the test baths. The preparations were either kept at rest or stimulated at 1 Hz. The release of $^{86}Rb^+$ into nonradioactive Tyrode's solution was then followed a) for 90 min under control conditions both at rest (45 min) and during stimulation (45 min) and b) for 90 min in the presence of forskolin 1 μmol/l, also at rest and during activity (same time protocol). The bath solution was changed every 15 min and collected in scintillation vials for later determination of radioactivity. At the end of the experiment, tissues were weighed and solubilized by the addition of 1 ml TS-1 (Zinsser, Frankfurt, FRG) and keeping them at 65° C for 3 h. Six milliliters of Minisolve (Zinsser) were added to each sample into the counting vials. Radioactivity was determined by liquid scintillation in a Tricarb 3380 counter (Packard Instruments, Frankfurt, FRG).

Measurement of $^{86}Rb^+$ uptake. It is known that Rb^+ and K^+ are transported similarly by the Na^+,K^+-exchange pump in the myocardium (Hougen et al. 1979; Akera et al. 1981). The uptake of $^{86}Rb^+$ is therefore an indirect measure of the activity of the Na^+,K^+-pump. For the determination of the pump activity, whole guinea-pig left atria were exposed to about 1 MBq $^{86}Rb^+$ for 1, 3, 10, 30 or 100 min, without or in the presence of forskolin 1 μmol/l. The preparations were kept at rest and allowed to stabilize in control or test solutions for 30 min. At the end of the incubation period, the muscles were treated for isotope analysis and radioactivity was determined as described above.

Chemicals. The following drugs were used (sources in parentheses): forskolin (Hoechst AG, Frankfurt, FRG); barium chloride (E. Merck, Darmstadt, FRG); atropine hydrochloride (Serva, Heidelberg, FRG); dimethyl sulfoxide, DMSO (Merck); ^{86}Rb chloride (New England Corp., Boston, MA, USA). All other chemicals (Merck) or as indicated. The stock solution of forskolin was dissolved in DMSO. The DMSO content of test solutions did not exceed 0.1% which by itself did not change action potential parameters.

Evaluation of results and statistical analyses. Results are either demonstrated as original figures or expressed as means ± standard error of the means (SEM). Action potential recordings were analyzed for maximal upstroke velocity (dV/dt_{max}), amplitude (APA), resting potential (RP), and duration (APD) at 20% and 90% of repolarization, APD_{20} and APD_{90}, respectively. Changes of the RP in the hyperpolarizing direction are described as an increase and in the depolarizing direction as a decrease in the RP. A single rate constant λ of $^{86}Rb^+$ efflux could be determined according to $\lambda = (\ln A_0 - \ln A)/t$ derived from $A = A_0\, e^{-\lambda t}$. When appropriate, statistically significant differences were assessed by Student's t-test or by analysis of variance (Wallenstein et al. 1980) followed by modified t-statistics according to Dunnett (1964). Significant differences are marked by one ($p < 0.05$) or two asterisks ($p < 0.01$).

Results

Figure 1 shows typical recordings of action potential and dV/dt in normal Tyrode's solution (A) and at 21.6 mmol/l KCl (B) both under control conditions and after the addition of forskolin. Under normal conditions, APD_{20} was increased by forskolin 1 μmol/l from 10 to 14 ms and APD_{90} was decreased from 82 to 60 ms; dV/dt_{max} remained

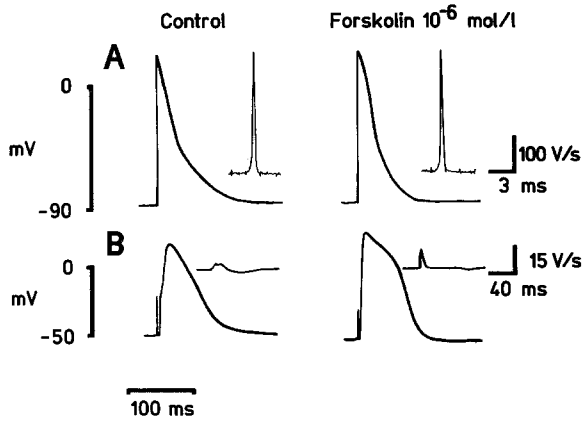

Fig. 1. Influence of forskolin on fast response **A** and slow response **B** action potentials in guinea-pig left atria. Original records of action potential and dV/dt under control conditions and 15 min after the addition of the drug

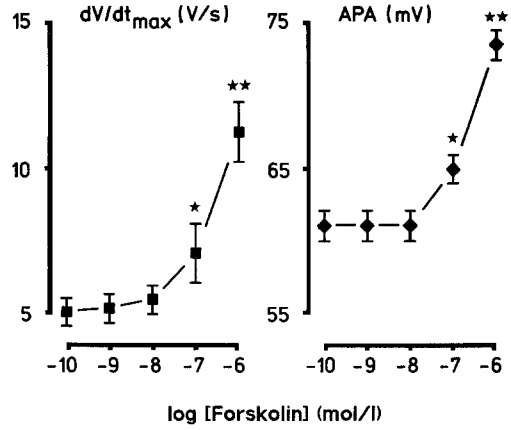

Fig. 3. Influence of forskolin on dV/dt_{max} and APA of slow response action potentials in guinea-pig left atria. Concentration-response relationships obtained by cumulative addition (every 10—20 min) of the drug. Means ± SEM of six preparations

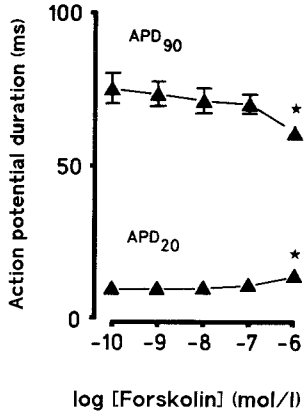

Fig. 2. Influence of forskolin on APD_{20} and APD_{90} in guinea-pig left atria. Concentration-response relationships obtained by cumulative addition (every 10—20 min) of the drug. Means ± SEM of six preparations

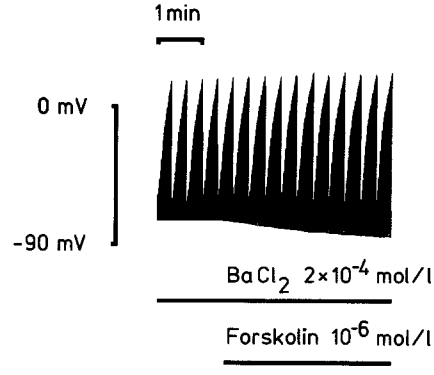

Fig. 4. Influence of forskolin on the RP in the presence of $BaCl_2$ in a guinea-pig left atrium. Originals records of 408 action potentials stored on a Nicolet digital scope (12 bit resolution). The different height of the action potentials is an artifact due to a phase transition between the driving frequency (2 Hz) of the analogue stimulator and the sample frequency (10 Hz) of the digital recording system

unchanged. At 21.6 mmol/l KCl, APA and duration of the slow response action potential were enlarged by forskolin; dV/dt_{max} was increased from 4.5 to 11 V/s. Figures 2 and 3 summarize all action potential measurements in guinea-pig left atrial preparations in response to cumulatively increasing concentrations of forskolin. Significant changes of the action potential parameters were generally obtained at 1 µmol/l forskolin. This concentration of forskolin was used for the determination of the effects of the drug on the RP and on $^{86}Rb^+$ fluxes.

Under normal conditions, an effect of a drug on the membrane RP into the hyperpolarizing direction is hardly detectable, because the value may be already near the equilibrium potential for potassium ions (Gelband et al. 1975). In further experiments, we determined the influence of forskolin on the RP previously diminished by the addition of $BaCl_2$ 0.2 mmol/l. Figure 4 depicts the original records of action potentials under control conditions (the RP was reduced by $BaCl_2$ from −87 to −74 mV) and after the addition of forskolin. Under these conditions, an increase in the overshoot from 16 to 23 mV and an increase in the RP from −74 to −84 mV were observed. Figure 5 summarizes the results obtained with forskolin without and

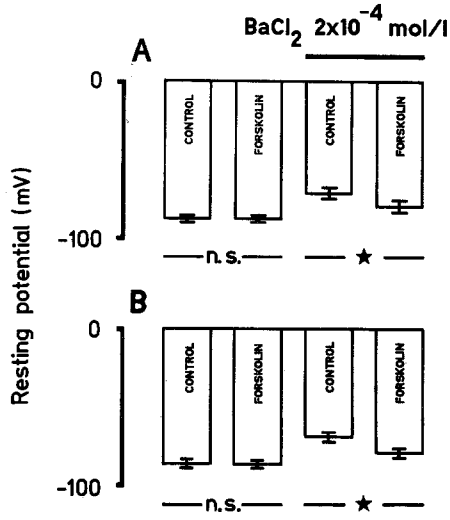

Fig. 5. Influence of forskolin (20 min after the addition) on RP in guinea-pig left atria without and in the presence of $BaCl_2$. **A** Without atropine; **B** in the presence of atropine 1 µmol/l. Means ± SEM of six preparations in each group

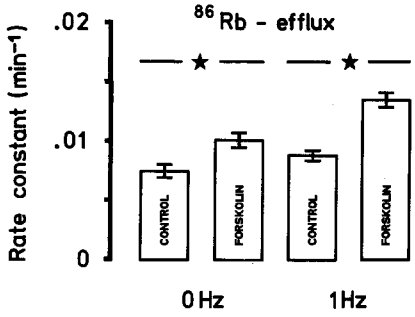

Fig. 6. Influence of forskolin 1 μmol/l on the rate constant of $^{86}Rb^+$ efflux in resting and in beating guinea-pig atria. Means ± SEM of 15 preparations. Rate constants were generally observed for 45 min

Table 1. Influence of forskolin 1 μmol/l on time-dependent $^{86}Rb^+$ uptake in resting guinea-pig left atria (cpm/100 mg wet weight). Means ± SEM; $n = 10$ in each field

	Control	Forskolin
1 min	1,619 ± 121	1,916 ± 250
3 min	4,196 ± 530	6,328 ± 1,011
10 min	10,259 ± 746	14,739 ± 3,414
30 min	25,105 ± 2,230	36,866 ± 4,109
100 min	66,056 ± 5,634	91,592 ± 9,643

Significance level: $p < 0.01$
(Two way-analysis of variance)

in the presence of $BaCl_2$. Note that first the RP was not significantly affected by forskolin in normal Tyrode's solution; second, in the presence of $BaCl_2$, there was a significant increase in the RP by about 9 mV in response to forskolin; third, qualitatively the same results were also obtained in the presence of atropine 1 μmol/l.

We have shown so far that forskolin increases the overshoot and the RP and decreases the duration of the action potential. It is not known why forskolin increases the RP and decreases the APD. We have therefore determined the rate constants of the $^{86}Rb^+$ efflux in resting and in beating preparations both under control conditions and in the presence of forskolin. It becomes clear from Fig. 6 that the efflux of $^{86}Rb^+$ is enhanced by forskolin in resting as well as in beating preparations. Figure 6 also demonstrates that the efflux of $^{86}Rb^+$ was slightly greater in beating than in resting preparations.

It seems also possible that forskolin stimulates the Na^+,K^+-pump and thereby increases the resting potential. To test this possibility, we have investigated the uptake of $^{86}Rb^+$ in resting preparations. Table 1 summarizes the results of the uptake measurements under control conditions and in the presence of forskolin 1 μmol/l. The table shows that the uptake of $^{86}Rb^+$ was significantly increased by forskolin at various incubation times.

Discussion

Forskolin has gained wide interest in both basic and clinical research for its unique action to directly activate the adenylate cyclase system, independent of any receptor activation, and to elevate cyclic AMP levels (Seamon and Daly 1986). Cyclic AMP levels are also increased by forskolin in isolated papillary muscles of the heart (Rodger and Shahid 1984). Forskolin was shown to mimic cyclic AMP-dependent actions on the heart including the positive inotropic and positive chronotropic effects (Lindner et al. 1978), increase in dV/dt_{max} of slow response action potentials (Späh 1984) and increase in I_{Ca} (Hescheler et al. 1986). Whereas Lindner et al. (1978), in the first report on the cardiac actions of forskolin, described a shortening of the action potential, a prolongation was described by Späh (1984) and by Rardon and Pappano (1986). It has been shown in the present paper that forskolin increases a potassium conductance at rest and in beating preparations and can either increase or decrease APD, dependent on the level of repolarization. Given the fact that forskolin also enhances I_{Ca} (Hescheler et al. 1986), the balance of inward and outward currents, which both are affected by forskolin, may well exert opposite effects on the APD under different experimental conditions.

The increase by forskolin of a potassium conductance not only shortens the APD but also causes a hyperpolarization which was demonstrated in the present study in preparations partially depolarized by $BaCl_2$. A possible contribution of muscarine receptors to the effect of forskolin was excluded in the present study (atropine did not prevent the hyperpolarization induced by forskolin). Agents which enhance I_{Ca} may secondarily increase outward currents. Two mechanisms may account for this effect. First, an increase in I_{Ca} will accentuate the plateau phase of the action potential which in turn activates more outward current (Kass and Tsien 1976). Second, an increase in $[Ca^{2+}]_i$ may increase the potassium conductance (Isenberg 1975). It seems, however, unlikely that the increase by forskolin in the potassium conductance is a secondary effect due to the enhanced I_{Ca}, since the effects were also seen in resting preparations, where the entry of calcium ions is supposed to be minimal.

It has been shown that the effects of cholinergic stimulation in the heart (Pfaffinger et al. 1985) and of dopamine, histamine and acetylcholine in ganglion cells (Sasaki and Sato 1987) are inhibited by pertussis toxin. It would therefore be of interest to determine whether or not a guanine nucleotide binding protein is involved in mediating the effects of forskolin on the potassium conductance. Forskolin was found to activate a Ca^{2+}-activated K^+-channel in cultured kidney cells (Guggino et al. 1985). On the other hand, direct anesthetic-like effects of forskolin on the nicotinic acetylcholine receptors of PC12 cells were described (McHugh and McGee 1986). In nerve cells or cultured nerve cell lines, forskolin increases the APD and decreases the potassium conductance (Dunlap 1985; Grega et al. 1987). Forskolin also blocked a potassium current in pancreatic B cells (Zünkler et al. 1987). These opposing results suggest that the effects of either forskolin or adenylate cyclase activation on the potassium conductance are by no means identical in different organ system.

The increase by forskolin of the RP could also result from a greater Na^+,K^+-ATPase pump current. Lindner et al. (1978) have shown that the ATPase activity of myocardial preparations is either not affected by forskolin or rather reduced at very high concentrations. However, since a cyclic AMP-dependent pump stimulation requires protein kinase-mediated phosphorylation, such an effect would not be detectable on isolated Na^+,K^+-ATPase. We have shown in intact preparations that the uptake of $^{86}Rb^+$ is increased by

forskolin which can be taken as indirect evidence for a greater pump activity.

Both effects of forskolin – increase in potassium conductance and stimulation of Na^+,K^+-pump – may be mediated by the accumulation of cyclic AMP. There is evidence that isoprenaline stimulates the Na^+,K^+-pump in isolated cardiac myocytes (Désilets and Baumgarten 1986). It has been shown earlier that adrenaline increases a voltage- and time-dependent outward current (Tsien et al. 1972; Brown et al. 1979). However, the final proof that cyclic AMP is the mediator of these effects of forskolin awaits further clarification.

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References

Akera T, Yamamoto S, Temma K, Kim D-H, Brody TM (1981) Is ouabain-sensitive rubidium or potassium uptake a measure of sodium pump activity in isolated cardiac muscle? Biochim Biophys Acta 640:779–790

Brown HF, DiFrancesco D, Noble SJ (1979) How does adrenaline accelerate the heart? Nature 280:235–236

Désilets M, Baumgarten CM (1986) Isoproterenol directly stimulates the Na^+-K^+ pump in isolated cardiac myocytes. Am J Physiol 251 (Heart Circ Physiol 20):H218–H225

Dunlap K (1985) Forskolin prolongs action potential duration and blocks potassium current in embryonic chick sensory neurons. Pflügers Arch 403:170–174

Dunnett CW (1964) New tables for multiple comparisons with a control. Biometrics 20:482–491

Ehara T, Inazawa M (1980) Calcium-dependent slow action potentials in potassium-depolarized guinea-pig ventricular myocardium enhanced by barium ions. Naunyn-Schmiedeberg's Arch Pharmacol 315:47–54

Gelband H, Myerburg RJ, Hoffman BF, Bassett AL (1975) Acetylcholine-induced reversal of canine and feline atrial myocardial depression during stretch, cardiac failure, and drug toxicity. Circ Res 37:542–549

Grega DS, Werz MA, MacDonald RL (1987) Forskolin and phorbol esters reduce the same potassium conductance of mouse neurons in culture. Science 235:345–348

Guggino SE, Suarez-Isla BA, Guggino WB, Sacktor B (1985) Forskolin and antidiuretic hormone stimulate a Ca^{2+}-activated K^+ channel in cultured kidney cells. Am J Physiol 249:F448–F455

Hescheler J, Kameyama M, Trautwein W (1986) On the mechanism of muscarinic inhibition of the cardiac Ca current. Pflügers Arch 407:182–189

Hougen TJ, Lloyd BL, Smith TW (1979) Effects of inotropic and arrhythmogenic digoxin doses and of digoxin-specific antibody on myocardial monovalent cation transport in the dog. Circ Res 44:23–31

Isenberg G (1975) Is potassium conductance of cardiac Purkinje fibres controlled by $[Ca^{2+}]_i$? Nature 253:273–274

Kass RS, Tsien RW (1976) Control of action potential duration by calcium ions in cardiac Purkinje fibers. J Gen Physiol 67:599–617

Lindner E, Dohadwalla AN, Bhattacharya BK (1978) Positive inotropic and blood pressure lowering activity of a diterpene derivative isolated from Coleus forskohlii: Forskolin. Arzneimittelforschung 28(I):284–289

McHugh EM, McGee R Jr (1986) Direct anesthetic-like effects of forskolin on the nicotinic acetylcholine receptors of PC12 cells. J Biol Chem 261:3103–3106

Metzger H, Lindner E (1981) The positive inotropic-acting forskolin, a potent adenylate cyclase activator. Arzneimittelforschung 31(II):1248–1250

Nawrath H, Blei I, Gegner R (1980) Opposite effects of β-adrenoceptor stimulation and 8-bromo-cyclic AMP on potassium efflux in mammalian heart muscle. Experientia 36:72–73

Pfaffinger PJ, Martin JM, Hunter DD, Nathanson NM, Hille B (1985) GTP-binding proteins couple cardiac muscarinic receptors to a K channel. Nature 317:536–538

Plant TD (1986) The effects of rubidium ions on components of the potassium conductance in the frog node of Ranvier. J Physiol 375:81–105

Rardon DP, Pappano AJ (1986) Carbachol inhibits electrophysiological effects of cyclic AMP in ventricular myocytes. Am J Physiol 251:H601–H611

Reuter H, Scholz H (1968) Über den Einfluß der extracellulären Ca-Konzentration auf Membranpotential und Kontraktion isolierter Herzpräparate bei graduierter Depolarisation. Pflügers Arch 300:87–107

Rodger IW, Shahid M (1984) Forskolin, cyclic nucleotides and positive inotropism in isolated papillary muscles of the rabbit. Br J Pharmacol 81:151–159

Sasaki K, Sato M (1987) A single GTP-binding protein regulates K^+-channels coupled with dopamine, histamine and acetylcholine receptors. Nature 325:259–262

Seamon KB, Daly JW (1986) Forskolin: Its biological and chemical properties. Adv Cyclic Nucleotide Protein Phosphorylation Res 20:1–150

Seamon KB, Padgett W, Daly JW (1981) Forskolin: Unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc Natl Acad Sci USA 78:3363–3367

Späh F (1984) Forskolin, a new positive inotropic agent, and its influence on myocardial electrogenic carbon movements. J Cardiovasc Pharmacol 6:99–106

Tsien RW, Giles W, Greengard P (1972) Cyclic AMP mediates the effects of adrenaline on cardiac Purkinje fibres. Nature 240:181–183

Wallenstein S, Zucker CL, Fleiss JL (1980) Some statistical methods useful in Circulation Research. Circ Res 47:1–9

Zünkler BJ, Ohno-Shosaku T, Trube G (1987) Forskolin blocks a K^+ current in pancreatic B cells. Naunyn-Schmiedeberg's Arch Pharmacol 335 (Suppl):R44

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