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Effects of levcromakalim and glibenclamide on paced guinea-pig atrial strips exposed to hypoxia

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Abstract

Isolated strips of guinea-pig atrial myocardium were mounted in isometric myographs and electrically paced for measurements of myocardial contractile function. Levcromakalim, a K^+ channel opener, completely inhibited the contractile force in a concentration-dependent way ($EC_{50} = 15$ μM). Glibenclamide (3 μM), a blocker of ATP-regulated K^+ channels (K_{ATP}), caused a 5-fold rightward shift of the concentration-effect curve. Exposure of the atrial strips to hypoxia caused a time-dependent loss of contractility from 100% to a minimum level of 60% within 12 min. Levcromakalim (1 μM, 3 μM and 10 μM) concentration-dependently enhanced the hypoxia-induced inhibition of contractile function whereas levcromakalim (0.01 μM and 0.1 μM) had no significant effect. In the presence of levcromakalim (10 μM) hypoxia reduced the contractile force to 25%. Glibenclamide (3 μM) totally antagonized the enhancing effect of levcromakalim. When hypoxia was induced in glucose-free Krebs solution with 2-deoxyglucose, the myocardial contractility was completely suppressed within 12 min. Glibenclamide by itself (3 μM) failed to influence the myocardial response to hypoxia both in normal Krebs solution and under conditions of impaired glycolysis. The results indicate that levcromakalim by activation of myocardial ATP-regulated K^+ channels accelerates and enhances the hypoxia-induced inhibition of myocardial contractile function. This effect may possibly contribute to the mechanism by which K^+ channel openers exert cardioprotection. The results further suggest that mechanisms different from activation of K_{ATP} take a major part in the depressant mechanical response to hypoxia and glycolytic blockade in the guinea-pig atrial myocardium.

Keywords: K^+ channel; Levcromakalim; Glibenclamide; Myocardium; Hypoxia; (Guinea-pig)

1. Introduction

K^+ channels in cardiac myocytes regulated by the intracellular ATP concentration (K_{ATP}) were first described by Noma (1983). The functional role of this channel may possibly be to protect the myocardium from damage caused by ischemia (Escande and Cavero, 1992). This view has been supported by the development of K_{ATP} activators and the demonstration that such drugs exert cardioprotective effects. Administration of K^+ channel openers like cromakalim, pinacidil, aprikalim and nicorandil before an ischemic insult in perfused ventricular walls (Cole et al., 1991), isolated perfused hearts (Grover et al., 1990) and also in vivo (Auchampach et al., 1991; Gross et al., 1992) improves postischemic contractile function, preserves intracellular ATP and reduces infarct size. The cardioprotection offered by these drugs can be reversed by the K_{ATP} blocker glibenclamide which, when given alone, aggravates ischemia-reperfusion damage and increases myocardial infarct size (Thornton et al., 1993). Ischemia, hypoxia, anoxia or treatment with blockers of glucose metabolism reduce intracellular ATP and thereby activate K_{ATP}. It is as yet not clear what is the precise mechanism by which activation of K_{ATP} protects the myocardium. The likely consequence of K_{ATP} opening in an ischemic region is a rapid arrest of myocardial contractile function due to K^+ efflux, early repolarisation, shortening of the action potential and inhibition of Ca^{2+} influx, which will tend to conserve energy and prolong survival of cardiac myocytes (Weiss et al., 1992). However, K_{ATP} are also present in the vascular smooth muscle cells of the coronary circulation and may possibly contribute to cardioprotection by causing vascular relaxation. Some K^+ channel openers

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have been demonstrated to selectively increase coronary collateral blood flow in ischemic areas (cf. Quast, 1993).

The aim of the present study was to investigate the possible role of K_{ATP} in the myocardial contractile response to hypoxia in isolated electrically paced guinea-pig atrial strips. Also the response to hypoxia under conditions of impaired glycolysis was studied. We also tested the hypothesis that pharmacological activation of the myocardial K_{ATP} channels would accelerate and enhance the loss of cardiac contraction during hypoxia. The non-perfused myocardial preparation used allows evaluation of myocardial responses to hypoxia and metabolic blockade without influence from effects mediated by K_{ATP} in coronary vessels. Levcromakalim and glibenclamide were chosen as a prototype activator and a blocker of K_{ATP}, respectively.

2. Materials and methods

2.1. Preparation of atrial strips and measurement of myocardial contractile function

Guinea-pigs of either sex (250–350 g) were killed by a blow to the neck. The heart was quickly excised and transferred to ice-cold oxygenated Krebs solution. The left atrium was isolated and strips of the pectinate muscle tissue (approximately $1 \times 0.5 \times 4$ mm) were prepared.

Two muscle preparations from each animal were transferred to two 5 ml tissue baths containing Krebs solution (36° C) gassed with a mixture of 95% O_2 and 5% CO_2. The composition of the Krebs solution was (in mM): NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15, glucose 5.5, pH = 7.4. The atrial strips were mounted in isometric precision myographs (Nielsen-Kudsk et al., 1986) for recording of mechanical activity. A Plexiglas cover was mounted on top of each tissue bath. In experiments involving hypoxia both the bathing solution as well as the local atmosphere under the Plexiglas seal were supplied with a mixture of 95% N_2 and 5% CO_2. The myocardial preparations were electrically paced at a frequency of 1 Hz with square wave impulses with a duration of 0.1 ms and a voltage amplitude 20% above threshold (Harvard Apparatus stimulator, model 345). The strips were gradually suspended to a passive load of 5 mN which in separate experiments was determined as optimal for generation of maximal active force. The amplified transducer signal was displayed on a Harvard recording system (modules 486 and 350) and a Siemens Elema Mingograph (model 82). The isometric contractile force and the maximal velocities of contraction and relaxation (first derivates of the contraction traces) were measured.

2.2. Experiments

The paced atrial strips were allowed to equilibrate for 90 min prior to experiments. Under conditions of normoxia, cumulative concentration-effect curves for levcromakalim were obtained in the absence or presence of glibenclamide (3 μM). Control preparations exposed to vehicle only were always run in parallel.

The myocardial response to hypoxia was recorded by abruptly shifting the aerating gas to a mixture of 95% N_2 and 5% CO_2. The parameters of myocardial contractile function were registered every 2 min for 30 min. The response to hypoxia was studied in the presence of levcromakalim (0.01 μM, 0.1 μM, 1 μM, 3 μM and 10 μM), glibenclamide (3 μM) or drug vehicle both in normal glucose-containing Krebs solution and in glucose-free Krebs solution containing 2-deoxyglucose (10 mM). The latter solution was used to block ATP production by glycolysis. Also the response to hypoxia in the simultaneous presence of both levcromakalim (10 μM) and glibenclamide (3 μM) was investigated. The drugs were added to the tissue baths 20 min before hypoxia. Samples of the Krebs solution of each tissue bath were analyzed for oxygen content at the end of every experiment (Radiometer oxygen electrode type E50146).

2.3. Data analysis

Results of measurements of contractile function during hypoxia are expressed relative to prehypoxic data as means ± S.E.M. Mean concentration-effect data for levcromakalim alone and in the presence of glibenclamide were fitted to the Hill function $E = 100 - (E_{MAX}C^S/(EC_{50}^S + C^S))$ by non-linear iterative regression analysis. Differences were evaluated by means of Student's t-test using a significance level of 5%.

2.4. Drugs

Drugs used were: glibenclamide (Hoechst, Germany), levcromakalim (a gift form SmithKline Beecham, UK) and 2-deoxyglucose (Sigma, USA). Glibenclamide (1 mM) was dissolved in 5% glucose containing NaOH (0.02 M), levcromakalim (10 mM) in 70% ethanol and 2-deoxyglucose (1 M) in saline 0.9%. Dilutions of levcromakalim were made in saline 0.9%.

3. Results

3.1. Effect of levcromakalim on myocardial contractile function

Levcromakalim caused a concentration-dependent inhibition of myocardial contractile force under condi-

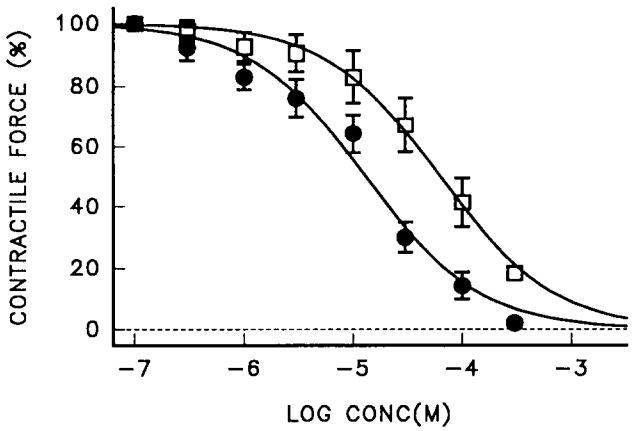

Fig. 1. Effect of levcromakalim on contractile force of isolated electrically paced guinea-pig atrial strips under conditions of normoxia. The concentration-effect curves were obtained in the absence (●, $n = 8$) or presence of glibenclamide (3 μM) (□, $n = 8$).

tions of normoxia. Concentration-response curves are shown in Fig. 1. Levcromakalim completely suppressed the contractile force with an inhibitory EC_{50} of 13 ± 5 μM, E_{max} of $100 \pm 9.5\%$ and a slope (S) of 0.84 ± 0.17. Glibenclamide (3 μM) shifted the concentration-effect curve for levcromakalim 5-fold to the right ($EC_{50} = 63 \pm 2$ μM) without changing the maximal effect of the drug. None of the vehicles influenced the contractility of the atrial strips.

3.2. Myocardial response to hypoxia and blockade of glycolysis

Shifting the aerating gas to a mixture of 95% N_2 and 5% CO_2 caused a fall of tissue bath pO_2 from above 500 mm Hg to 18.6 ± 1.3 mm Hg within 2 min. The myocardial response of the electrically paced atrial

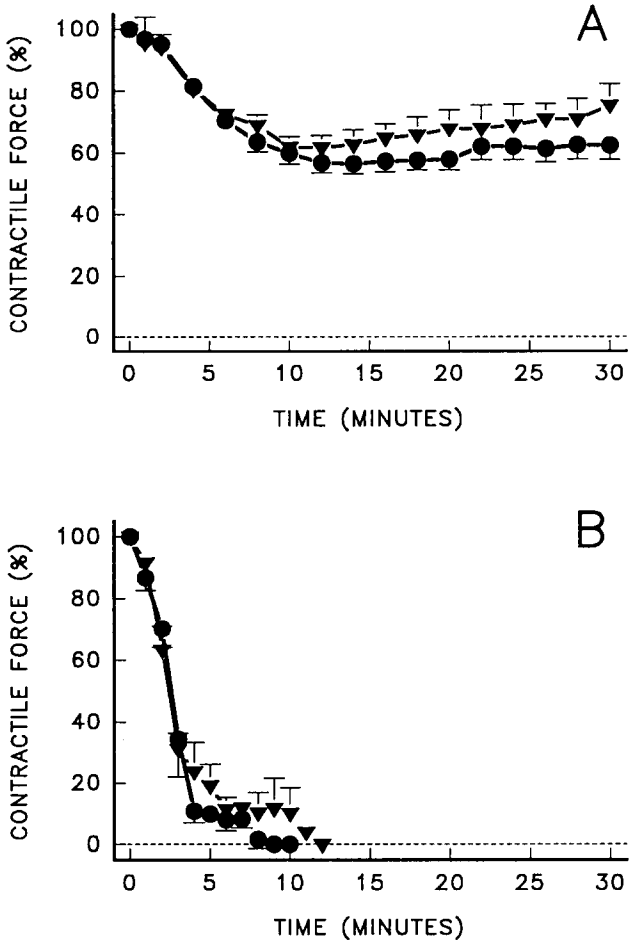

Fig. 2. A: Myocardial contractile response to hypoxia of isolated electrically paced guinea-pig atrial strips in normal Krebs solution and B: in glucose-free Krebs solution containing 2-deoxyglucose. The curves were obtained either in the presence of vehicle only (●; A: $n = 29$, B: $n = 10$) or glibenclamide (3 μM) (▼; A and B: $n = 10$).

Table 1
Effects of hypoxia on contractile force and velocities of contraction and relaxation of isolated electrically paced strips of the guinea-pig atrial myocardium

	Contractile force (%)	Contraction velocity (%)	Relaxation velocity (%)
Vehicle ($n = 29$)	56.8 ± 3.3	54.2 ± 2.8	48.1 ± 2.8
Levcromakalim (10 μM, $n = 8$)	24.9 ± 5.2 [a]	24.9 ± 3.6 [a]	20.4 ± 3.4 [a]
Levcromakalim (3 μM, $n = 6$)	32.7 ± 4.3 [a]	31.1 ± 3.6 [a]	24.3 ± 3.2 [a]
Levcromakalim (1 μM, $n = 8$)	41.3 ± 4.4 [a]	42.3 ± 4.2 [a]	35.3 ± 3.8 [a]
Levcromakalim (0.1 μM, $n = 8$)	48.8 ± 6.7	45.9 ± 5.5	43.2 ± 7.8
Levcromakalim (0.01 μM, $n = 6$)	54.1 ± 5.9	50.0 ± 5.1	45.9 ± 5.4
Glibenclamide (3 μM, $n = 10$)	61.9 ± 3.8	58.1 ± 3.4	55.3 ± 2.8
Levcromakalim (10 μM) + glibenclamide (3 μM, $n = 9$)	56.7 ± 5.6	55.4 ± 4.9	49.8 ± 4.4

The data were obtained 12 min after onset of hypoxia (maximal effects) in the presence of either vehicle only, levcromakalim, glibenclamide or levcromakalim and glibenclamide together. Data are expressed as mean percentages ± S.E. of the absolute values measured just before exposure to hypoxia: 2.6 ± 0.5 mN (contractile force), 80 ± 13 mN/s (velocity of contraction) and 44 ± 7 mN/s (velocity of relaxation). A normal glucose-containing Krebs solution was present in the tissue baths. [a] $P < 0.05$ compared to vehicle.

strips to hypoxia is shown in Fig. 2A. Hypoxia caused a significant time-dependent decline of the myocardial contractile force to a minimum level of 56.8 ± 3.3% which occurred within 12 min (100% is the level of contractile force before onset of hypoxia). At the same time hypoxia reduced the velocity of contraction to 54.2 ± 2.8% and the velocity of relaxation to 48.1 ± 2.8%. No further suppression of contractile force was seen from 12 to 30 min after the onset of hypoxia. Table 1 summarizes the changes of the measured dynamic parameters after 12 min of hypoxia.

The response to hypoxia under conditions of impaired glycolysis is shown in Fig. 2B. Hypoxia produced a fast and complete inhibition of the contractile activity of the guinea-pig atrial myocardium. The contractile force was reduced to less than 10% within 5 min and was completely suppressed after 12 min. The response to hypoxia was recorded 20 min after shifting the bathing solution to a glucose-free Krebs solution with 2-deoxyglucose. The shift did not influence the myocardial contractile function.

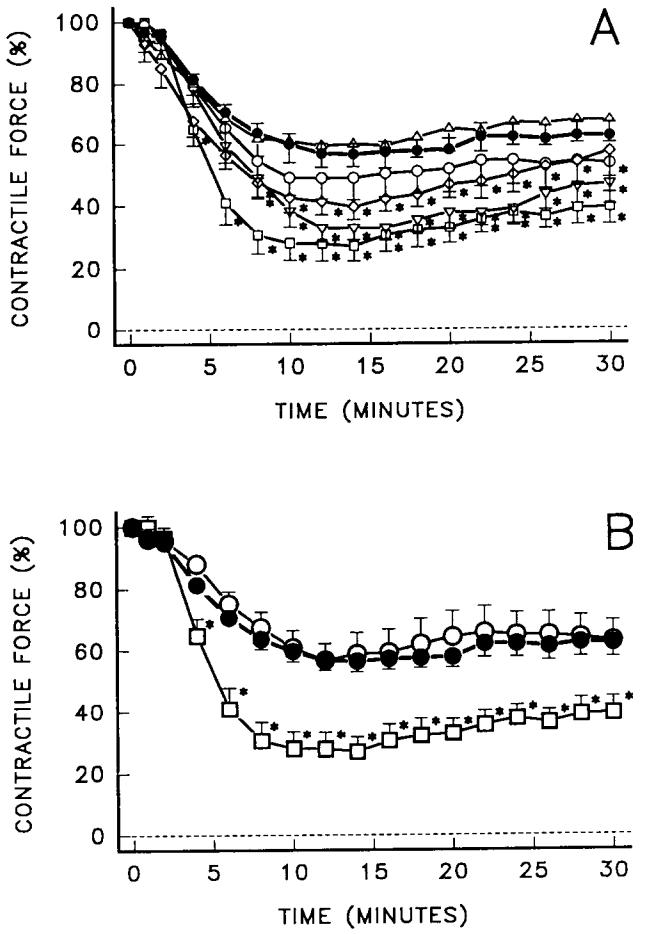

Fig. 3. A: Effect of levcromakalim 0.01 μM (△), 0.1 μM (○), 1 μM (◇), 3 μM (▽) and 10 μM (□) on the hypoxia-induced inhibition of contractile function of isolated electrically paced strips of the guinea-pig atrial myocardium ($n = 6$–8). Strips which were exposed to vehicle only are represented by • ($n = 29$). B: Complete reversal by glibenclamide (3 μM) of the myocardial action of levcromakalim (10 μM) in paced guinea-pig atrial strips exposed to hypoxia. Atrial strips treated by vehicle (•, $n = 29$), levcromakalim (□, $n = 8$) or glibenclamide + levcromakalim (○, $n = 9$). *Significant difference from vehicle treated strips ($P < 0.05$).

3.3. Effects of levcromakalim and glibenclamide on the myocardial response to hypoxia and blockade of glycolysis

Levcromakalim (1 μM, 3 μM and 10 μM) caused a significant concentration-dependent enhancement of the hypoxia-induced inhibition of myocardial contractile function (Fig. 3A). Lower concentrations of levcromakalim (0.01 μM and 0.1 μM) had no significant enhancing effect. In the absence of levcromakalim (vehicle present) the myocardial contractile force was reduced to 56.8 ± 3.3% whereas at the highest concentration of levcromakalim (10 μM) hypoxia reduced the myocardial contractile force to 24.9 ± 5.2%. Also the hypoxia-induced inhibition of the velocity of contraction and relaxation was augmented by levcromakalim (Table 1). Glibenclamide (3 μM) totally reversed the enhancing effect of levcromakalim (10 μM) (Fig. 3B).

Glibenclamide (3 μM) by itself did not significantly influence the myocardial mechanical response to hypoxia (Fig. 2A and Table 1). Also higher concentrations of glibenclamide (10 and 30 μM, data not shown) failed to influence the contractile parameters during hypoxia. When hypoxia was induced under conditions of impaired glycolysis, glibenclamide (3 μM) again failed to significantly modify the myocardial response (Fig. 2B).

4. Discussion

Levcromakalim exerted a concentration-dependent negative inotropic action under normoxic conditions which is in accordance with previously reported results obtained in the isolated guinea-pig ventricular and atrial myocardium (Shigenobu et al., 1991; Lau, 1992). Glibenclamide (3 μM) caused a 5-fold rightward shift of the concentration-effect curve for levcromakalim without suppression of the maximal effect, which indicates a competitive type of interaction between the two drugs. The results suggest that the myocardial inhibitory effect of levcromakalim is mediated by activation of K_{ATP}.

When the atrial strips were exposed to hypoxia, a gradual reduction of contractile force and velocities of contraction and relaxation was observed. During the experimental period of hypoxia from the 12th to the 30th minute the strips stabilized at approximately 60% of the prehypoxic level of contractile force. This suggests that during hypoxia a significant non-oxidative energy production takes place in the guinea-pig atrial myocardium. This is supported by our finding of a

complete inhibition of myocardial contractile function under conditions where hypoxia was combined with exposure to a glucose-free medium with 2-deoxyglucose, which blocks glycolysis. The results indicate that both the oxidative and the glycolytic pathways are essential for generation of ATP in the guinea-pig atrial myocardium.

The K_{ATP} blocker glibenclamide failed to inhibit the myocardial response to hypoxia. Weiss and Lamp (1987) found that in single guinea-pig ventricular myocytes formation of ATP via the glycolytic pathway was more effective than ATP formation via oxidative metabolism in preventing opening of K_{ATP}. However, in the present study glibenclamide also under conditions of glycolytic blockade failed to modulate the hypoxic myocardial response. Although we observed a slight (but insignificant) tendency towards an inhibitory effect of glibenclamide on contractile activity at the end of the experimental period of hypoxia in both media, our findings suggest either that activation of K_{ATP} is not a major mechanism by which hypoxia suppresses myocardial contractile function or that glibenclamide is a weak or insufficient blocker of K_{ATP} under hypoxic conditions. Venkatesh et al. (1991) found that the blocking effect of glibenclamide on K_{ATP} in single rabbit ventricular cells was greatly inhibited in the presence of ADP at the cytosolic membrane surface. This finding could imply that the binding affinity of glibenclamide is impaired under conditions of hypoxia where the intracellular concentration of ADP is likely to increase. This phenomenon could theoretically contribute to the lack of significant influence of glibenclamide on the loss of cardiac contraction during hypoxia in the present study. However, we demonstrated that glibenclamide was an effective blocker when the myocardial K_{ATP} channels were activated by levcromakalim under hypoxic conditions. The present negative findings for glibenclamide suggest that mechanisms different from activation of K_{ATP} mediate the hypoxia-induced inhibition of myocardial contractile function. Yan et al. (1993) recently showed that glibenclamide only partially affected the K^+ loss in perfused hypoxic rabbit papillary muscle which suggested that other ion channels may possibly be involved in the response to hypoxia besides the K_{ATP} channel. High energy phosphate can be rapidly exhausted during hypoxia and most likely contributes to the reduction of mechanical activity. Accumulation of intracellular metabolites such as inorganic phosphate and H^+ may decrease the Ca^{2+} sensitivity of the myofilaments and thereby serve as mediators of hypoxic contractile dysfunction (Koretsune and Marban, 1990; Ikenouchi et al., 1991).

Our results obtained with levcromakalim demonstrate a concentration-dependent enhancement of the effect of hypoxia on myocardial contractile function. The action of levcromakalim (10 μM) was fully antagonized by glibenclamide (3 μM), which indicates that opening of K_{ATP} is the underlying mechanism. Since hypoxia by itself can be expected to activate the channel, levcromakalim seems in some way to potentiate the effects of decreased intracellular concentration of ATP. Thuringer and Escande (1989) found that the action of the K^+ channel opener RP 49356 on single myocardial cells was due to decreased sensitivity of K_{ATP} to the blocking action of ATP, possibly because of competition between the drug and ATP at the receptor site. Although ischemia is a more complex event than experimental hypoxia, it is possible that K_{ATP} activators like levcromakalim may enhance the inhibition of myocardial mechanical activity induced by an ischemic insult in vivo. A rapid and pronounced suppression of myocardial contraction in the ischemic region would conserve energy and prolong survival of cardiomyocytes. Enhancement of the negative inotropic response to hypoxia as demonstrated with levcromakalim may possibly contribute to the mechanism of cardioprotection by K_{ATP} activators. In a recent study by Yao and Gross (1994) it was shown that the K_{ATP} activator, bimakalim, at two doses that markedly accelerated the action potential reduced myocardial infarct size in anesthetized open-chest dogs. Interestingly, a low dose that did not significantly influence the action potential duration exerted the same degree of cardioprotection indicating that the electrophysiological effects of K^+ channel openers can possibly be dissociated from their cardioprotective properties. However, this study does not rule out that the mechanism for the cardioprotective effect was an acceleration and enhancement of the loss of contractility in the ischemic myocardium and in the myocardial areas adjacent to the region of infarction. This would require measurements of regional wall motion. The authors measured dP/dt but this only reflects the global function of the left ventricle. In addition a clear dissociation between cellular K^+ loss, tissue ATP levels and action potential shortening during hypoxia was demonstrated in the study by Yan et al. (1993). A recent presentation argues against cardioplegia as the mechanism by which K_{ATP} openers produce cardioprotection. In the guinea-pig right ventricular wall perfused via the coronary artery the K_{ATP} opener aprikalim was reported to produce cardioprotection with an accelerated decrease in action potential duration, but without enhancement of the rate of ischemia-induced loss of myocardial contractility (Djellas et al., 1993).

Acknowledgements

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References

Auchampach, J.A., M. Maruyama, I. Cavero and G.J. Gross, 1991, The new K^+ channel opener aprikalim (RP 52891) reduces experimental infarct size in dogs in the absence of hemodynamic changes, J. Pharmacol. Exp. Ther. 259, 961.

Cole, W.C., C.D. McPherson and D. Sontag, 1991, ATP-regulated K^+ channels protect the myocardium against ischemia/reperfusion damage, Circ. Res. 69, 571.

Djellas, Y., M. Mestre and I. Cavero, 1993, Aprikalim protection against ischemic injury occurs with an accelerated decrease in action potential duration but not in myocardial contractility, Circulation 88 I-632, abstract 3399.

Escande, D. and I. Cavero, 1992, K^+ channel openers and 'natural' cardioprotection, Trends. Pharmacol. Sci. 13, 269.

Gross, G.J., J.A. Auchampach, M. Maruyama, D.C. Warltier and G.M. Pieper, 1992, Cardioprotective effects of nicorandil, J. Cardiovasc. Pharmacol. 20 (Suppl. 3), S22.

Grover, G.J., S. Dzwonczyk, C.S. Parham and P.G. Sleph, 1990, The protective effects of cromakalim and pinacidil on reperfusion function and infarct size in isolated perfused rat hearts and anesthetized dogs, Cardiovasc. Drugs Ther. 4, 465.

Ikenouchi, H., O. Kohmoto, M. McMillan and W.H. Barry, 1991, Contributions of $[Ca^{2+}]_i$, $[Pi]_i$ and pH_i to altered diastolic myocyte tone during partial metabolic inhibition, J. Clin. Invest. 88, 55.

Koretsune, Y. and E. Marban, 1990, Relative roles of Ca^{2+}-dependent and Ca^{2+}-independent mechanisms in hypoxic contractile dysfunction, Circulation 82, 528.

Lau, W.-M., 1992, Effects of potassium channel blockers on the negative inotropic responses induced by cromakalim and pinacidil in guinea pig atrium, Pharmacology 45, 9.

Nielsen-Kudsk, F., B. Poulsen, C. Ryom and J.E. Nielsen-Kudsk, 1986, A strain-gauge myograph for isometric measurements of tension in isolated small blood vessels and other muscle preparations, J. Pharmacol. Methods 16, 215.

Noma, A., 1983, ATP-regulated K^+ channels in cardiac muscle, Nature 305, 147.

Quast, U., 1993, Potassium channel openers: pharmacological and clinical aspects, Fundam. Clin. Pharmacol. 6, 279.

Shigenobu, K., C. Kageyama and M. Watanabe, 1991, Action potential shortening and negative inotropic effects of a novel potassium channel opener, NIP-121, as compared with cromakalim in guinea pig ventricular myocardium, Jpn. J. Pharmacol. 57, 117.

Thornton, J.D., C.S. Thornton, D.L. Sterling and J.M. Downey, 1993, Blockade of ATP-sensitive potassium channels increases infarct size but does not prevent preconditioning in rabbit hearts, Circ. Res. 72, 44.

Thuringer, D. and D. Escande, 1989, The potassium channel opener RP 49356 modifies the ATP-sensitivity of K^+-ATP channels in cardiac myocytes, Pflüg. Arch. 414 (Suppl. 1), S175.

Venkatesh, N., S.T. Lamp and J.N. Weiss, 1991, Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle, Circ. Res. 69, 623.

Weiss, J.N. and S.T. Lamp, 1987, Glycolysis preferentially inhibits ATP-sensitive K^+ channels in isolated guinea pig cardiac myocytes, Science 238, 67.

Weiss, J.N., N. Venkatesh and S.T. Lamp, 1992, ATP-sensitive K^+ channels and cellular K^+ loss in hypoxic and ischaemic mammalian ventricle, J. Physiol. (London) 447, 649.

Yan, G.-X., K.A. Yamada, A.G. Kleber, J. McHowat and P.B. Corr, 1993, Dissociation between cellular K^+ loss, reduction in repolarization time, and tissue ATP levels during myocardial hypoxia and ischemia, Circ. Res. 72, 560.

Yao, Z. and G.J. Gross, 1994, Effects of the K_{ATP} channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs, Circulation 89, 1769.