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European Journal of Pharmacology 437 (2002) 165–171

www.elsevier.com/locate/ejphar

Selective mitochondrial K_{ATP} channel activation results in antiarrhythmic effect during experimental myocardial ischemia/reperfusion in anesthetized rabbits

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Received 3 January 2002; accepted 11 January 2002

Abstract

We investigated the effects of administration of non-hypotensive doses of ATP-sensitive K^+ channel (K_{ATP}) openers (nicorandil and aprikalim), and a specific mitochondrial K_{ATP} channel blocker (5-hydroxydecanoate) prior to and during coronary occlusion as well as prior to and during post-ischemic reperfusion on survival rate, ischemia/reperfusion-induced arrhythmias and myocardial infarct size in anesthetized albino rabbits. Arrhythmias were induced by reperfusion following a 20 min ligation of the left main coronary artery with a releaseable silk ligature. Early intervention by intravenous infusion of nicorandil (100 μg/kg bolus + 10 μg/kg/min) or aprikalim (10 μg/kg bolus + 0.1 μg/kg/min) just before and during ischemia increased survival rate (86% and 75% vs. 55% in the control group), significantly decreased the incidence and severity of life-threatening arrhythmias and myocardial infarct size. The antiarrhythmic and cardioprotective effects of both nicorandil and aprikalim were abolished by pretreating the rabbits with 5-hydroxydecanoate (5 mg/kg, i.v. bolus). In conclusion, intervention by intravenous administration of nicorandil and aprikalim (through the selective activation of mitochondrial K_{ATP} channels) increased survival rate and exhibited antiarrhythmic and cardioprotective effects during coronary occlusion and reperfusion in anesthetized rabbits when administered prior to and during coronary occlusion.

Keywords: Nicorandil; Aprikalim; K_{ATP} channel, mitochondrial; (Anesthetized rabbit); Coronary occlusion; Myocardial ischemia; Reperfusion arrhythmia

1. Introduction

Both sarcolemmal and mitochondrial K_{ATP} channels in the cardiovascular system might have a physiological role in modulating cardiac function, particularly under conditions of metabolic stress, such as hypoxia, ischemia, and metabolic inhibition when intracellular ATP is reduced. During myocardial ischemia, as the intracellular ATP concentration falls and ischemic metabolites (ADP, lactate, H^+) accumulate, the probability of the channel being open increases. This leads to an enhanced outward repolarizing flow of K^+ and hyperpolarization of the cell membrane. As a consequence, the myocardial action potential duration is shortened, the voltage-dependent calcium current and myocardial contractility are decreased leading to ATP preservation during ischemia thereby helping in exerting a protective property during myocardial ischemia/reperfusion (Fujita and Kurachi, 2000; Nichols and Lederer, 1991).

Recent studies hint that mitochondrial K_{ATP} channel opening rather than sarcolemmal (surface) K_{ATP} channel opening may play a dominant role in affording cardioprotection in ischemic/reperfused hearts. The K_{ATP} channel from cardiac myocyte mitochondria has been reported to be 2000 fold more sensitive to diazoxide than the channel from cardiac myocyte sarcolemma, indicative of the fact that two distinct receptor subtypes coexist within the myocyte (Liu et al., 1998; Garlid et al., 1996, 1997). Reports indicate that nicorandil, a hybrid K_{ATP}-channel opener and nitrate compound (Taira, 1989) in lower doses exerts a direct cardioprotective effect on heart muscle cells, an effect mediated by the selective activation of mitochondrial K_{ATP} channels (Sato et al., 2000a,b). Nevertheless, Critz et al.(1997) reported that nicorandil caused neither sarcolemmal K_{ATP} channel opening nor cardioprotection in rabbit cardiomyocytes.

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01277-3

K_{ATP} channel openers have been reported to be proarrhythmic associated with a tendency to increase infarct size during acute ischemia/reperfusion in vitro in isolated, perfused heart preparations in many studies (DiDiego and Antzelevitch,1993; Fagbemi et al.,1993; Tosaki et al., 1993; Kitzen et al.,1992). Other studies have yielded contradictory results, suggesting that such agents can preserve myocardial function and decrease infarct size (Yao and Gross, 1994; Auchampach and Gross, 1994), as well as produce an antiarrhythmic effect (Baczko et al., 1997) by inducing opening of sarcolemmal K_{ATP} channels. Moreover, the relative contributions of sarcolemmal and mitochondrial K_{ATP} channel modulation on arrhythmias during acute myocardial ischemia and reperfusion are unclear.

The aims of our present work, therefore, were (i) to assess whether treatment with non-hypotensive doses of K_{ATP} channel openers nicorandil and aprikalim, the selective mitochondrial K_{ATP} channel blocker 5-hydroxydecanoate, as well as 5-hydroxydecanoate/nicorandil and 5-hydroxydecanoate/aprikalim produce proarrhythmic or antiarrhythmic effects, and whether opening of the sarcolemmal or mitochondrial K_{ATP} channels is relatable to this effect, and (ii) to find out whether nicorandil or aprikalim given prior to and during coronary occlusion or prior to and during reperfusion result in cardioprotection, in a well-standardized model of arrhythmias in anesthetized rabbits, during reperfusion.

2. Materials and methods

2.1. Animals

Male New Zealand White rabbits, weighing between 2 and 3 kg were used in the experiments. The care and use of animals in this work were in accordance with "Guidelines for the Care and Use of Laboratory Animals", prepared by the Indian National Science Academy, New Delhi (Anonymous, 2000) and the European Community guidelines for the use of experimental animals. The experimental protocol was approved by the institutional ethics committee.

2.2. Surgical preparation of the animals and hemodynamic measurements

Rabbits were anesthetized with pentobarbitone sodium (30 mg/kg) and ketamine hydrochloride (35 mg/kg) administered via a marginal ear vein. After the trachea was cannulated via a midline cervical incision, animals were ventilated with room air supplemented with O_2, and tidal volume was adjusted as necessary throughout the experimental procedure to maintain arterial pH between 7.3 and 7.5, pO_2 at >20 kPa and pCO_2 at <5 kPa. The rate of ventilation was fixed at 56 cycles/min. The right jugular vein was cannulated with polyethylene tubing for intravenous administration of drugs or saline.

Blood pressure was measured from the right common carotid artery using a pressure transducer (P23XL; Gould, Valley View, OH, USA) for continuous recording of arterial blood pressure as well as intermittent arterial blood gas measurements (AVL 993;AVL Medical Instruments UK, Staffs, UK).Rectal temperature was monitored periodically and maintained at 38.5 ± 0.5 °C by a heating pad.

The thorax was opened in the left 4th intercostal space and after pericardiotomy the heart was exposed. A loose loop of 3–0 atraumatic silk (NW 5070, Ethicon, Johnson & Johnson, India) was placed around the left main coronary artery about 2 mm from its origin. The ends of the silk were passed through the wall of a cylinder-shaped polyethylene tube formed from a cannula and were led outside the thorax. Thereafter, the heart was set back in its place.

Electrodes were attached to the shaved area on each limb for recording the surface electrocardiogram (ECG). After allowing time (approximately 5 min) for the blood pressure and the heart rate to stabilize the coronary artery was occluded by pulling the ends of the silk ligature taut to produce myocardial ischemia. Myocardial ischemia was confirmed by visible regional epicardial cyanosis, regional hypokinesis within 20–30 s and ECG changes (increase in R wave and ST segment elevation). Reperfusion was effected by releasing the silk ligature whence visible hyperemia was evident on the surface.

2.3. Survival and electrocardiographic arrhythmia recordings

The ECG was continuously recorded using limb lead II. The survival rate and the duration of arrhythmias were registered in accordance with the Lambeth Conventions (Walker et al., 1988), i.e., ventricular fibrillation, ventricular tachycardia and other various arrhythmias like single ventricular ectopic beats, bigeminia and salvos. When ventricular fibrillation occurred, no attempt was made to defibrillate the animals.

An arrhythmia score was used to evaluate the incidence and duration of various arrhythmias by giving a grade to each animal as follows: 0 = no arrhythmias; 1 = < 10 s ventricular tachycardia or other arrhythmias, no ventricular fibrillation; 2 = 11–30 s ventricular tachycardia or other arrhythmias, no ventricular fibrillation; 3 = 31–90 s ventricular tachycardia or other arrhythmias, no ventricular fibrillation; 4 = 91–180 s ventricular tachycardia or other arrhythmias, and/or <10s reversible ventricular fibrillation; 5 = 180 s ventricular tachycardia or other arrhythmias, and/or >10 s reversible ventricular fibrillation; 6 = irreversible ventricular fibrillation (Lepran et al., 1996).

2.4. Experimental protocol and drug treatment

The experiment involved 145 rabbits allocated into 12 groups of 12, 8, 8, 12, 13, 14, 13, 10, 12, 14, 15 and 14, respectively, and the protocols are summarized in Fig. 1. All

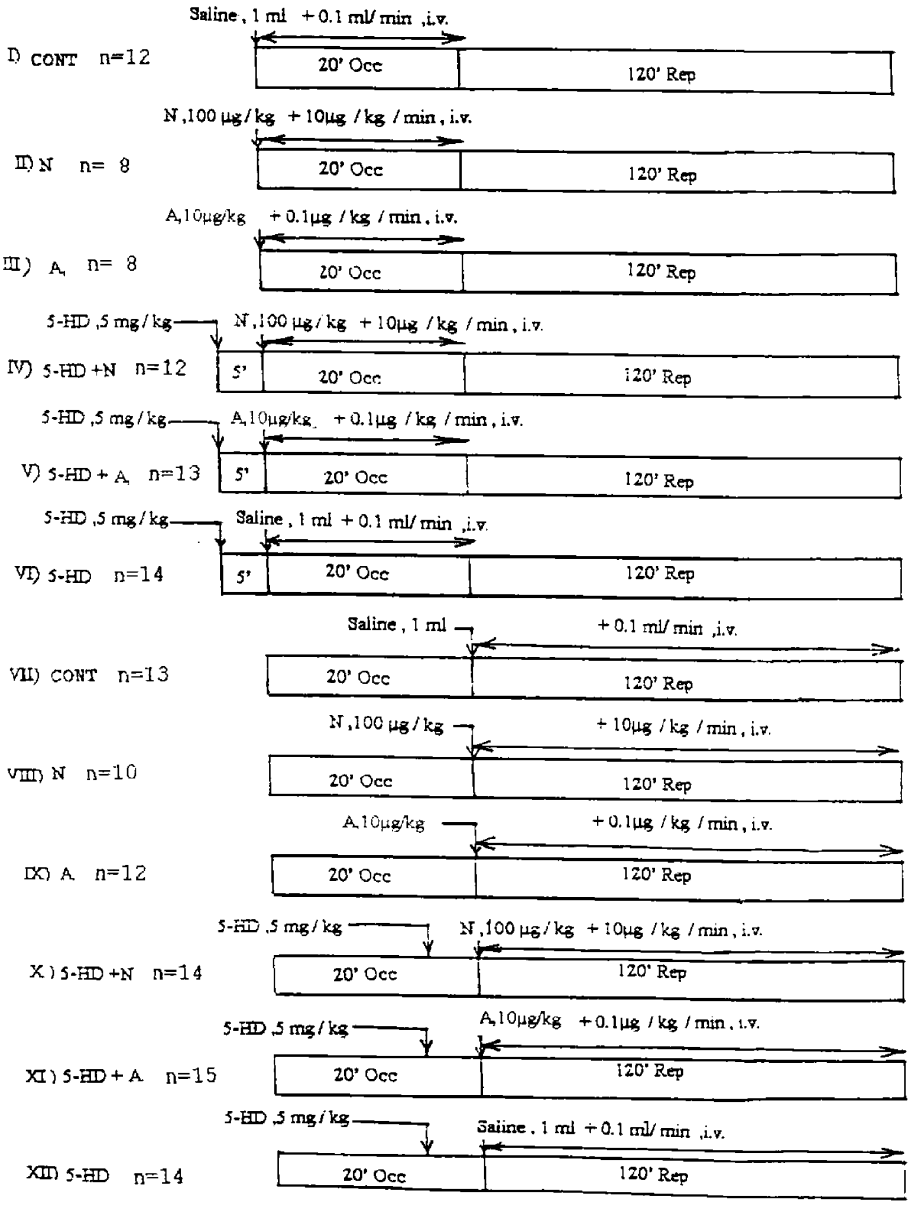

Fig. 1. Experimental protocol. Occ = period of coronary occlusion; Rep = period of reperfusion; CONT = control group; N = Nicorandil; A = Aprikalim; 5-HD + N = 5-hydroxydecanoate/Nicorandil; 5-HD + A = 5-hydroxydecanoate/Aprikalim; 5-HD = 5-hydroxydecanoate; i.v. = intravenous injection.

the animals were subjected to a 20-min coronary occlusion followed by 120 min of reperfusion. In case of ventricular fibrillation at the time of onset of reperfusion, the experiment was terminated and the animal was considered dead during ischemia and was excluded from the study.

The animals in Groups I and VII acted as the control group. Animals in Groups II and III were used to detect the ability of treatment with nicorandil and aprikalim, respectively, during the period of coronary occlusion on ischemia/reperfusion-induced arrhythmias and myocardial infarct size. The animals in Groups IV and V were used to determine the effects of treatment with nicorandil and aprikalim, respectively, during the period of coronary occlusion on reperfusion-induced arrhythmias and myocardial infarct size in rabbits pretreated with 5-hydroxydecanoate. The animals in Group VI were used to determine the effect of pretreatment with 5-hydroxydecanoate before ischemia on ischemia/reperfusion-induced arrhythmias and myocardial infarct size. Animals in Groups VIII and IX were used to detect the ability of pretreatment with nicorandil and aprikalim, respectively, during the period of reperfusion on ischemia/reperfusion-induced arrhythmias and myocardial infarct size. Animals in Groups X and XI were used to determine the effects of treatment with nicorandil and aprikalim, respectively, during the period of reperfusion on reperfusion-induced arrhythmias and myocardial infarct size in rabbits

pretreated with 5-hydroxydecanoate. Animals in Group XII were used to determine the effect of pretreatment with 5-hydroxydecanoate before reperfusion.

2.5. Determination of the infarct size

After completing all measurements at the end of each experiment, staining of the risk area was accomplished by reoccluding the coronary artery and injecting methylene blue (3%) into the venous cannula thereby demarcating the area at risk. After 3 min, the heart was excised from the body of the animal and washed in isotonic saline. After freezing, the heart was sliced transversely from apex to base in 2 mm sections. The slices were defrosted, blotted and then incubated at 37 °C with 0.1% wt./vol. nitroblue tetrazolium in Sorensen's phosphate buffer (0.1M, pH 7.4) for 30 min. Normal myocardium was stained dark blue within 15 min, while the infarcts (necrotic tissues) remained unstained or stained only faintly. The areas of risk and infarct were then determined by using a computer-aided planimetry.

2.6. Drugs

We obtained nicorandil pure powder as a gift from Torrent Pharmaceuticals (Gujarat, India) and aprikalim from Rhone Poulenc (Mumbai, India). We purchased methylene blue and nitroblue tetrazolium from Sigma (St. Louis, MO, USA) and 5-hydroxydecanoate (sodium salt) from Research Biochemicals International (Natick, MA, USA).

2.7. Statistical analysis

For the statistical analysis of the survival rate, percentage protection and the incidence of arrhythmias (percentage), Fischer's Exact Probability Test was applied. Arrhythmia scores were compared using Kruskal–Wallis Rank Sum Test. The other parameters were expressed as Mean ± S.E.M. and after one-way analysis of variance (one-way ANOVA) were compared by means of Duncan's New Multiple Range Procedure. The null hypothesis was rejected at $P < 0.05$.

3. Results

3.1. Hemodynamic variables (heart rate, mean arterial blood pressure (MABP)) and differences in arterial blood gases

There were no significant differences in these hemodynamic parameters between groups throughout the experimental period (i.e., baseline values were the same for each group). Rectal temperature and arterial blood pH were well-maintained in the physiological range, and there were no significant differences between groups during the course of experimentation. Arterial pO_2 and pCO_2 levels were maintained between 20–60 and 3.5–4.5 kPa, respectively, in all the 12 groups.

3.2. Arrhythmias during reperfusion

Following the 20-min occlusion of the coronary artery and the resulting regional myocardial ischemia and infarction, the silk ligature around the artery was released for reperfusion to occur. Reperfusion arrhythmias started within 30–45 s in the control animals. More number of animals survived the reperfusion period in the groups (II and III) which were treated with nicorandil and aprikalim, respectively, during the coronary occlusion (75% and 75% vs. 50%, P = not significant) but not in the groups (VIII and IX) which received nicorandil and aprikalim, respectively, during the period of reperfusion compared to the control animals (Table 1).

The effects of nicorandil, aprikalim, 5-hydroxydecanoate/nicorandil, 5-hydroxydecanoate/aprikalim and 5-hydroxydecanoate alone on the incidence and severity of arrhythmias during the 120 min of reperfusion are presented in Table 1. During reperfusion, the number of animals without any arrhythmias were significantly more in groups II and III, i.e., the animals which were treated with nicorandil and aprikalim, respectively, during the period of ischemia due to coronary occlusion but not in groups VIII and IX which were treated with nicorandil and aprikalim,

Table 1
Effects of nicorandil, aprikalim, 5-hydroxydecanoate/nicorandil, 5-hydroxydecanoate/aprikalim and 5-hydroxydecanoate alone on the incidence of arrhythmias during reperfusion in anesthetized rabbits

Group	n	Survived (No./%)	Arrhythmia incidence (No./%)				Mean arrhythmia score[a]
			None	VF	VT	Other	
Drugs administered prior to and during coronary occlusion							
I(CONT)	12	6/50	0/0	5/42	12/100	12/100	5.8 ± 0.3
II(NICO)	8	6/75	5/63[b]	0/0	1/13[b]	2/25[b]	0.6 ± 0.1[b]
III(APR)	8	6/75	5/63[b]	1/13	1/13[b]	2/25[b]	1.0 ± 0.2[b]
IV(5-HD + NIC)	12	6/50	1/8	6/50	11/92	12/100	5.5 ± 0.9
V(5-HD + APR)	13	6/46	2/15	8/62	11/85	13/100	5.7 ± 1.1
VI(5-HD)	14	6/43	1/7	9/64	14/100	14/100	5.8 ± 0.6
Drugs administered prior to and during reperfusion							
VII(CONT)	13	6/46	0/0	8/62	11/85	12/92	5.9 ± 0.5
VIII(NICO)	10	6/60	2/20	5/50	8/80	10/100	5.1 ± 0.5
IX(APR)	12	6/50	2/16	7/58	9/75	11/92	5.2 ± 0.3
X(5-HD + NIC)	14	6/43	3/21	8/57	13/93	12/88	5.8 ± 0.8
XI(5-HD + APR)	15	6/40	1/7	10/67	13/87	14/93	5.6 ± 0.7
XII(5-HD)	14	6/43	6/43	11/79	13/93	13/93	5.8 ± 0.9

n = Total number of animals; No. = number of animals exhibiting the given response; % = percentage of the animals exhibiting the given response; VF = ventricular fibrillation; VT = ventricular tachycardia; Other = extrasystoles, salvos and/or bigeminy; CONT = Control; NICO = Nicorandil; APR = Aprikalim; 5-HD = 5-hydroxydecanoate.

[a] Data are mean ± S.E.M.

[b] $P < 0.05$ vs. control.

respectively, during reperfusion. 5-Hydroxydecanoate pretreatment abolished the beneficial effects of both nicorandil and aprikalim on reperfusion arrhythmias.

Treatment with nicorandil and aprikalim, respectively, during the period of coronary occlusion in groups II and III decreased the incidence of ventricular fibrillation, ventricular tachycardia and other arrhythmias in comparison to the control group (Group I) or groups pretreated with 5-hydroxydecanoate. Statistically significant decreases in the incidence of ventricular tachycardia in Group II (13% vs. 100%, $P<0.05$) and Group III (13% vs. 100%, $P<0.05$) as well as other arrhythmias in Group II (25% vs. 100%, $P<0.05$) and Group III (25% vs. 100%, $P<0.05$) were observed in comparison with the control animals.

The arrhythmia scores which take into account the severity and the duration of arrhythmias in addition to the survival rate in a single numerical quantity were also significantly decreased in Group II animals and Group III animals (Table 1).

Statistically significant reductions in the lengths of arrhythmic attacks were obtained in Groups II and III (which received nicorandil and aprikalim, respectively, during coronary occlusion) for ventricular fibrillation (0.0 ± 0.0 and 1.1 ± 1.1 min vs. 20.4 ± 8.2 min, $P<0.01$), for ventricular tachycardia (1.6 ± 1.6 and 3.6 ± 3.6 min vs. 83.1 ± 16.1 min, $P<0.01$) and for total duration of arrhythmic attacks (5.0 ± 3.1 and 9.4 ± 4.2 min vs. 108.6 ± 21.3 min, $P<0.01$). No reduction in the lengths of arrhythmic attacks were seen in Groups VIII and IX which received nicorandil and aprikalim, respectively, during the period of reperfusion.

3.3. Myocardial infarct size

All the experimental animals clearly demonstrated areas of myocardial infarction arising from 20 min of coronary occlusion and 120 min of reperfusion. The area at risk was not significantly different among the various groups. A significant reduction in myocardial infarct size, expressed as a percentage of the area at risk were observed in Groups II and III only, i.e., in those animals which received nicorandil and aprikalim, respectively, during the 20 min coronary occlusion period ($21 \pm 2\%$ and $21 \pm 3\%$ vs. $45 \pm 5\%$, $P<0.01$) compared to the control group. The cardioprotective effects of nicorandil and aprikalim (when administered to rabbits during coronary occlusion, i.e., before reperfusion) were abolished by pretreatment with the selective mitochondrial K_{ATP} channel blocker, 5-hydroxydecanoate. However, by itself it appears that treatment with 5-hydroxydecanoate seems to have little effect on myocardial infarct size.

4. Discussion

The present study demonstrates that pretreatment with nicorandil and aprikalim, both K_{ATP} channel openers infused prior to and during the period of coronary occlusion (but not prior to and during reperfusion) may offer significant protective effect during reperfusion in anesthetized rabbits. Nicorandil and aprikalim treatment significantly decreased the incidence of life-threatening arrhythmias like sustained ventricular fibrillation and ventricular tachycardia. Nicorandil and aprikalim were quite effective in increasing the number of animals that survived without developing any arrhythmia during reperfusion. On the other hand, 5-hydroxydecanoate, a specific mitochondrial K_{ATP} channel blocker offered no protection against arrhythmias in the ischemic-reperfused rabbit heart in vivo. However, pretreatment with 5-hydroxydecanoate abolished the beneficial effects of nicorandil and aprikalim on ischemia/reperfusion-induced arrhythmias and cardioprotection suggesting that such effects may have probably been achieved via the selective opening of mitochondrial K_{ATP} channels rather than sarcolemmal K_{ATP} channels.

Nicorandil, a hybrid ATP-dependent potassium channel (K_{ATP}) opener and nitrate compound (Taira, 1987, 1989), is being used clinically for the treatment of angina pectoris (Krumenacker and Roland, 1992). The cardioprotective effects of nicorandil in ischemic hearts have received utmost attention as nicorandil can improve the recovery of postischemic contractile dysfunction, can reduce infarct size and can exert antiarrhythmic action in several animal models (Gross, 1993; Mizumura et al., 1995; Imagawa et al., 1998; Das et al., 2001a,b) and in humans (Kobayashi et al., 1998; Ito et al., 1999; Patel et al., 1999). Aprikalim is a tetrahydrothiopyran capable of opening K_{ATP} channels (Gross and Auchampach,1992). K_{ATP} channel openers may shorten the action potential duration, thereby reducing cellular calcium overload and preserving viability in ischemic/reperfused myocardium: this was initially proposed as the mechanism for protection of ischemic myocardium. However, this hypothesis cannot account for the mechanism of cardioprotection, because abbreviation of action potentials is not necessary for protection (Yao and Gross, 1994; Grover et al., 1995, 1996). Alternatively, recent pharmacologic evidence hints that mitochondrial K_{ATP} channels are the dominant effectors. The relatively selective K_{ATP} channel opener, diazoxide protects rabbit ventricular myocytes in a cell pelleting model of ischemia (Liu et al., 1998) and improves functional recovery after ischemia in isolated rat and rabbit hearts (Garlid et al., 1997); this diazoxide-induced protection is abrogated by 5-hydroxydecanoate (Garlid et al., 1997; Liu et al., 1998) which is a selective mitochondrial K_{ATP} channel blocker.

Interestingly, Sakai et al. (1999, 2000) reported subcellular localization of nicorandil in cardiomyocyte mitochondrial fractions of rats after oral dosing, where nicorandil is partly transformed into SG-86 (denitrated nicorandil) with release of nitric oxide (NO). NO has been implicated as a mediator of "second window" ischemic preconditioning and mitochondrial K_{ATP} channels are the likely effectors. NO directly activates mitochondrial K_{ATP} channels and

potentiates the ability of nicorandil to open these channels (Sasaki et al., 2000).

Several potential mechanisms may be considered as reasons for the beneficial effects of nicorandil and aprikalim. The existence in mitochondria of separate, highly regulated pathways for K^+ efflux and influx stongly implies that mitochondrial volume is subject to regulation in vivo. Volume, in turn has been shown to regulate activity of the electron transport chain. Mitochondrial K_{ATP} channels by controlling the mitochondrial K^+ cycle are thought to be involved with mitochondrial volume control and cellular bio-energetics (Garlid, 1996). Most likely, reperfusion provides oxygen to reactivate mitochondrial respiration but also causes a large production of oxygen free radicals (as the electron transport chain is in a reduced state) and a large influx of Ca^{2+} in the cytosol as a result of sarcolemmal damage. Mitochondrial Ca^{2+} transport is, therefore, stimulated at maximal rates. Mitochondrial Ca^{2+} accumulation causes profound alterations in the permeability of the inner membrane to solutes, leading to severe mitochondrial swelling. In addition, Ca^{2+} transport takes precedence over ATP synthesis and hence energy production. Thus, it is likely that mitochondrial K_{ATP} activation may act to inhibit ATP waste. Nicorandil in low doses (100 μM) has been recently reported to open only mitochondrial K_{ATP} channels but not sarcolemmal (surface) K_{ATP} current, whereas 10-fold higher concentrations recruit (open) both mitochondrial K_{ATP} and sarcolemmal K_{ATP} channels. Pooled dose–response data have confirmed that nicorandil concentrations as low as 10 μM turn on mitochondrial K_{ATP} channels, while sarcolemmal K_{ATP} current requires exposure to millimolar concentrations. Recently, Shinbo and Iijima (1997) reported that the K_{ATP} current induced by K_{ATP} channel openers is potentiated by NO in guinea-pig ventricular cells. It has been found that orally administered nicorandil distributes into heart mitochondria where it is partly transformed into mainly its denitrated compound, SG-86, possibly leading to continuous release of NO. Thus, it is also possible that the mitochondrial K_{ATP} channel-opening activity of nicorandil and SG-86 may be enhanced by the released NO in mitochondria. However, NO and NO donors (e.g., *S*-nitroso-*N*-acetyl-DL-penicillamine) only weakly favour the opening of mitochondrial K_{ATP} channels and it seems unlikely that the cardioprotective effect of nicorandil is solely conferred by its nitrate moiety (Sasaki et al., 2000). Interestingly, in in vitro cell-pelleting models of ischemia, a potent sarcolemmal K_{ATP} channel blocker did not block cardioprotective effects of nicorandil (Sato et al., 2000a,b). In contrast, in our set of experiments, 5-hydroxydecanoate, which is a selective mitochondrial K_{ATP} channel blocker, abolished the beneficial effects of nicorandil and aprikalim on the rabbit myocardium. Thus, one possible mechanism for myocardial protection during reperfusion by K_{ATP} channel openers, e.g., nicorandil and aprikalim, may be by influencing intracellular calcium-controlling mechanisms, possible through mitochondrial K_{ATP} channels (Piper et al., 1988; Sato et al., 2000a,b; Sakai et al.,2000). Hence, the older hypothesis that the "antiarrhythmic" effect accruing out of the decrease in voltage-dependent calcium influx secondary to improved repolarization and action potential duration shortening mediated by sarcolemmal K_{ATP} channel opening by K_{ATP} channel openers (Baczko et al.,1997; Brooks et al., 1995) may not be appropriate in this context.

Despite their favourable cardioprotective property, enthusiasm for K_{ATP} channel openers has been tempered by the fear that they may promote the development of/predispose to ventricular arrhythmias (Chi et al., 1990).This potential drawback limits the clinical utility of sarcolemmal K_{ATP} channel openers. In contrast, more selective mitochondrial K_{ATP} channel openers protect cardiac myocytes from ischemia/reperfusion-induced injury (Garlid et al., 1997; Liu et al., 1998; Baines et al., 1999) and arrhythmias (Patel et al., 1999; Das et al., 2001a,b, in press), suggesting that mitochondrial K_{ATP} channels might be useful targets for protection against ischemia/reperfusion-induced injury and consequent lethal ventricular arrhythmias. These results suggest receptor or channel subtypes. The differences in drug sensitivity and subcellular localization indicate that mitochondrial K_{ATP} channels are distinct from surface K_{ATP} channels at a molecular level (Grover and Garlid, 2000; Hu et al., 1999). In this study, we have found that non-hypotensive doses of nicorandil and aprikalim appear to be fairly selective mitochondrial K_{ATP} channel openers.

To conclude, the present study suggests that treatment with non-hypotensive doses of nicorandil or aprikalim prior to and during coronary occlusion may result in protection against ischemia/reperfusion-induced arrhythmias, reduce myocardial infarct size and increase survival during acute myocardial infarction in anesthetized rabbits most probably by opening mitochondrial K_{ATP} channels in the myocardial cells.

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