Effect of combined $K_{ATP}$ channel activation and $Na^+/H^+$ exchange inhibition on infarct size in rabbits

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Hale, Sharon L., and Robert A. Kloner. Effect of combined $K_{ATP}$ channel activation and $Na^+/H^+$ exchange inhibition on infarct size in rabbits. Am J Physiol Heart Circ Physiol 279: H2673–H2677, 2000.—We tested if combining treatment with cariporide, an $Na^+/H^+$ exchange inhibitor, and diazoxide, a mitochondrial ATP-sensitive $K^+$ ($K_{ATP}$) channel opener, would reduce myocardial infarct size (IS) to a greater extent than either intervention alone. Four groups of rabbits were studied ($n = 10$ each): cariporide (0.3 mg/kg), diazoxide (10 mg/kg), both drugs, and saline control, given 15 min before a 30-min coronary artery occlusion and 3 h reperfusion. IS in controls comprised $47 \pm 6\%$ of the risk region. Cariporide reduced IS by $55\%$ compared with control ($21 \pm 3\%$), but diazoxide did not significantly reduce IS compared with controls ($37 \pm 6\%$). Combined treatment resulted in an IS of $18 \pm 5\%$. Also we determined that diazoxide did not potentiate a subthreshold dose of cariporide nor did a mitochondrial $K_{ATP}$ channel blocker, 5-hydroxydecanoate (5-HD), prevent cariporide from reducing IS. Thus cariporide reduced necrosis by $>50\%$ in this model, both in the presence and absence of $K_{ATP}$ channel blockade. There was no significant difference in IS reduction between the group receiving cariporide alone and the group receiving combined treatment. Because the effect of cariporide was not blocked by 5-HD, it is unlikely that $K_{ATP}$ channels play a role as an end effector in cariporide’s mechanism.

INHIBITION of the $Na^+/H^+$ exchanger has been observed to produce cardioprotection in many animal models (6, 10, 11, 13, 15). Inhibition of this pathway with drugs such as diazoxide has been shown to reduce necrosis both when given before the onset of ischemia (6, 11) and when given later at the time of reperfusion (9, 18). Drugs that prevent $Na^+$ entry into the cell may protect against reperfusion injury by preventing calcium overload in the cells. Although inhibition of the $Na^+/H^+$ exchanger mimics ischemic preconditioning, it has been shown that the cardioprotective effect is not protein kinase C mediated and is thus probably not related to ischemic preconditioning (15, 19).

Diazoxide, a benzothiadiazine, is a specific activator of mitochondrial ATP-sensitive $K^+$ channels ($K_{ATP}$). Activation of mitochondrial $K_{ATP}$ channels is thought to provide cardioprotection as one of the end effectors of ischemic preconditioning. Opening of these channels before the onset of ischemia, by agents such as diazoxide, has been shown by some investigators to reduce myocardial necrosis (2, 4, 5, 16, 17, 25).

The differing actions of these two pathways suggested the possibility that the inhibition of the $Na^+/H^+$ exchanger and the activation of mitochondrial $K_{ATP}$ channels may be additive and together increase resistance against myocardial ischemia. Thus the purpose of this study was to investigate the potential of combined treatment with cariporide and diazoxide to reduce infarct size after coronary artery occlusion and reperfusion in the rabbit model.

Initial data from our study showed that the inhibition of the $Na^+/H^+$ exchanger was indeed cardioprotective in our model, whereas activation of mitochondrial $K_{ATP}$ channels failed to reduce infarct size compared with the control group. When the two interventions were combined, there was no further reduction in infarct size than was obtained with inhibition of the $Na^+/H^+$ exchanger alone. To further elucidate these two pathways, two additional groups were studied. One group received the mitochondrial $K_{ATP}$ channel blocker 5-hydroxydecanoate (5-HD) and the $Na^+/H^+$ exchange inhibitor cariporide. If the final mechanism by which cariporide reduced infarct size was through activation of the $K_{ATP}$ channels, then it would be expected that cariporide would be ineffective in reducing infarct size in the presence of 5-HD. To test whether opening of $K_{ATP}$ channels could potentiate the effect of cariporide, the second group received a subthreshold dose of cariporide together with the mitochondrial $K_{ATP}$ channel activator diazoxide.

METHODS

The rabbits used in this study were maintained in accordance with the policies and guidelines of the position of the American Heart Association on research animal use (1) and Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The rabbit model of coronary artery occlusion/reperfusion is a standard model for assessing myocardial infarct size and is characterized by little to no coronary collateral flow within the ischemic bed (14).

**Preparation.** Male New Zealand White rabbits weighing 1.8–3.0 kg were anesthetized with an intramuscular injection of ketamine (200 mg) and xylazine (100 mg) given twice before the start of surgery. To maintain a deep level of anesthesia, pentobarbital sodium anesthesia was given as needed throughout the protocol. The rabbits were intubated and respired using room air supplemented with 100% oxygen. Fluid-filled catheters were inserted into the jugular vein to give fluids and into the carotid artery to monitor arterial blood pressure. After the chest and pericardium were opened, a large branch of the circumflex artery was encircled with a small atraumatic needle attached to 4-0 silk suture. The needle was removed, and the ends of the suture were threaded through tubing, forming a snare, which was used to occlude and reperfuse the artery. A catheter was inserted into the left atrial appendage through which blue dye was injected at the end of the protocol to outline the ischemic risk region. Body temperature in all rabbits was between 38 and 39°C at the time of treatment and coronary artery occlusion. Heart rate and blood pressure were measured at baseline (before treatment), after 15 min of treatment (just before occlusion), at 25 min of occlusion, and at various time points during reperfusion.

**Experimental protocol.** After a 15-min stabilization period, the rabbits were randomly assigned to treatment groups. Four groups were studied (n = 10 in each group). One group received cariporide (0.3 mg/kg), one group received diazoxide (10 mg/kg), one group received both drugs, and one group acted as control receiving only saline. Treatment was given intravenously 15 min before the onset of ischemia. All rabbits then received 30 min of coronary artery occlusion followed by 3 h of reperfusion. At the end of the protocol, the coronary artery was reoccluded and 4 ml of 50% Uniserse blue (Ciba-Geigy, Hawthorne, NY) were injected into the left atrial catheter and allowed to circulate throughout the vasculature to delineate the risk region (unstained by blue dye). The rabbit was euthanized by an overdose of xylazine (300 mg iv) followed by 12 meq of potassium chloride given into the left atrium. The ischemic risk region and the region of necrosis, delineated with triphenyltetrazolium chloride staining, were measured as previously described (8).

**Additional study.** To further elucidate an interaction between these two pathways, an additional 14 rabbits were randomly assigned to two added groups. One group received a subthreshold dose of cariporide, 0.01 mg/kg (13), 20 min before coronary occlusion followed by diazoxide (10 mg/kg). To determine whether cariporide acted independently of opening of K$_{ATP}$ channels, the second group received the K$_{ATP}$ channel blocker 5-HD (5 mg/kg) 20 min before coronary artery occlusion followed 5 min later by cariporide (0.3 mg/kg).

**End points and data analyses.** The end points measured were mean arterial blood pressure, heart rate, area at risk, and infarct size. Mean arterial pressure was calculated by the formula: [(systolic pressure − diastolic pressure)/3] + diastolic pressure. All data summary and statistical analyses were performed using SAS (version 6.14, Cary, NC). Left ventricular weights, infarct size, area at risk, and baseline body temperatures were compared using one-way ANOVA. If an f < 0.05 was obtained, differences among the means were determined by Tukey’s test. Heart rate and blood pressure were analyzed by repeated-measures ANOVA. Differences among the blood pressure means were analyzed with ANOVA at specific time points if the overall value of f exceeded f < 0.05. Data are expressed as means ± SE.

**RESULTS**

Forty-two rabbits entered the first protocol. Two rabbits, both in the control group, died of ventricular fibrillation during the occlusion period. Data are reported on the remaining 40 rabbits, 10 per group. In the second study, 14 rabbits were entered. One rabbit was excluded because the risk region was <10% of the left ventricle. Data are reported on seven hearts in the diazoxide + low-dose cariporide group and six hearts in the cariporide + 5-HD group. All groups had similar mean body weights, left ventricular weights, and body temperatures at the onset of coronary artery occlusion (data not shown).

**Heart rate and mean arterial pressure.** Heart rates and mean arterial pressures in the first four groups are shown in Fig. 1. Heart rates were similar in all groups at baseline (169 to 176 beats/min), and there were no significant changes from baseline or among groups throughout the protocol. Diazoxide reduced mean arterial pressure compared with control after treatment but before coronary artery occlusion (P < 0.05); during occlusion and reperfusion pressures in all groups decreased from baseline (P < 0.0001 for group effect), however, pressures in the treatment groups were not significantly different from control. In the second study, diazoxide treatment reduced mean arterial pressure from 97 ± 3 mmHg before treatment to 68 ± 15 min after treatment. Heart rates and arterial pressures during the remainder of the protocol were similar to those of the initial study in both groups.

**Risk zone and infarct size.** The ischemic risk zone, expressed as a percent of the left ventricle, was not significantly different among the initial four groups (Fig. 2, top) and on average comprised ~30–36% of the
left ventricle. Despite similar risk zones, mean infarct sizes in the groups receiving cariporide and combined cariporide + diazoxide therapy were significantly smaller compared with the control group, but diazoxide did not significantly reduce infarct size compared with controls nor was combined therapy significantly better than cariporide alone. Large symbols represent means ± SE for each group. * P < 0.01 vs. control.

In the second study, the risk zone averaged 33 ± 2% of the left ventricle in cariporide + 5-HD group and 24 ± 4% in the diazoxide + low-dose cariporide group. Infarct size in the diazoxide + low-dose cariporide group was 38 ± 8% of the risk region, comparable to that observed in the initial study. Infarct size in the cariporide + 5-HD group was 11 ± 3% of the risk region. Thus inhibition of the mitochondrial K_{ATP} channels using 5-HD did not affect the protectiveness of cariporide.

**DISCUSSION**

The aim of this study was to test the effect of combined treatment with an Na^+/H^+ exchange inhibitor and a K_{ATP} channel opener on the development of necrosis occurring after coronary artery occlusion and reperfusion in the rabbit. Singly, both of these interventions have been shown to be cardioprotective in many animal models, but their combined use as a therapeutic intervention has yet to be tested.

Cariporide is a selective Na^+/H^+ exchange subtype 1 inhibitor, which has been shown to be cardioprotective in various experimental models. For example, in rabbits, it reduces infarct size in a dose-dependent fashion when given as a pretreatment (13). In swine, it reduces functional impairment caused by ischemia (22), and, in rats, it decreases arrhythmias after coronary artery occlusion and reperfusion (21) and attenuates apoptosis (3). Briefly, the role of the Na^+/H^+ exchanger is to maintain intracellular pH and to control calcium and sodium ion homeostasis (12). During ischemia, with the development of intracellular acidosis, the exchanger is activated to move H^+ out of the cell and to bring Na^+ in. As ischemia progresses, the cell becomes overloaded with Na^+, the Na^+/Ca^2+ exchanger is activated, and calcium ions enter the cell. The calcium overload that can occur is thought to be associated with irreversible cell damage. Although it has been suggested that sodium-potassium exchange inhibition is a pathway of ischemic preconditioning, recent studies indicate that this is probably not the case (15, 20).
The second drug tested in this study was diazoxide, a specific activator of mitochondrial ATP-sensitive K⁺ channels. Early work by Yao and Gross (24) showed that opening \( K_{\text{ATP}} \) channels using bimakalim lowered the time threshold for ischemic preconditioning in dogs. In addition, these investigators found that the opening of \( K_{\text{ATP}} \) channels was a necessary part of the “memory” of ischemic preconditioning when the interval between the preconditioning episode and the sustained ischemia was prolonged (26). Activation of mitochondrial \( K_{\text{ATP}} \) channels is now thought to be one of the end effectors of ischemic preconditioning. Opening of these channels before the onset of ischemia by agents such as diazoxide has been shown by some investigators to increase the time to onset of contracture, improve functional recovery (4), and reduce infarct size in rats (23) and rabbits (2, 4, 16, 17). This mechanism also has positive effects when initiated after the onset of ischemia. When a potassium channel opener was infused into the coronary artery of dogs during the first 10 min of a 1-h coronary artery occlusion, infarct size was reduced (25).

Data from the present study confirms that pretreatment with the sodium/hydrogen exchange inhibitor cariporide reduces necrosis in the rabbit model of coronary artery occlusion and reperfusion. We observed a pronounced reduction in infarct size (55% compared with control) as reported in studies by Miura et al. (15) and Linz et al. (13). There was no significant difference in the infarct size reduction between the group that received cariporide alone and the group that received both cariporide and diazoxide, suggesting that combined therapy did not provide better protection that giving cariporide alone.

A surprising finding was that, in contrast with some other investigators (2, 17), we did not find that treating rabbits with diazoxide significantly reduced infarct size compared with control in either study. We did observe a negative correlation in both studies between the reduction in mean arterial pressure caused by diazoxide treatment and the size of the infarct. This observation raises the possibility that in some in vivo preparations, diazoxide might be contributing to alterations in infarct size by mechanisms other than opening \( K_{\text{ATP}} \) channels within the myocardium. Perhaps a drop in blood pressure causes brief transient global coronary hypoperfusion which then induces ischemic preconditioning in some rabbits. Or perhaps this drop in pressure triggers reflex sympathetic tone or catecholamine release that could act as a preconditioning mimetic factor. Other possible reasons for the difference between our study and others are presently unknown. The dose of diazoxide used in our study was 10 times higher that that used by Ockaili et al. (16), who administered the drug 30 min before ischemia, but was identical to that used by Baines et al. (2), who gave the drug 10 min before ischemia.

Inasmuch as opening \( K_{\text{ATP}} \) channels has been shown to lower the threshold of the timing of ischemic preconditioning (24), we tested whether diazoxide administration and the subsequent opening \( K_{\text{ATP}} \) channels could potentiate a subtherapeutic dose of cariporide. We chose the dose of 0.01 mg/kg cariporide, because this had previously been shown in a dose-response study to be the highest dose that failed to reduce infarct size in the rabbit model (13). Diazoxide failed to potentiate this dose of cariporide, and infarct size in this study (38 ± 8% of the risk zone) was similar to that in our first study (37 ± 6% of the risk region, \( P = \text{NS} \) compared with control hearts).

It is possible that cariporide might exert a positive effect “upstream” of the \( K_{\text{ATP}} \) channels. To test this, the \( K_{\text{ATP}} \) channel blocker 5-HD was given before cariporide. 5-HD did not inhibit the infarct size-reducing capability of cariporide, and, in fact, infarct size in the cariporide + 5-HD group was lower than when cariporide alone was given.

In summary, the Na⁺/H⁺ exchange inhibitor cariporide reduced the size of myocardial infarction by >50% in this experimental model. Diazoxide treatment did not significantly reduce infarct size compared with control. There was no significant difference in the infarct size reduction between the group that received cariporide alone and the group that received both cariporide and diazoxide, suggesting that combined therapy did not provide better protection than giving cariporide alone. Diazoxide failed to potentiate a subtherapeutic dose of cariporide, and blockade of \( K_{\text{ATP}} \) channels had no effect on the infarct reducing capability of cariporide. Thus it is unlikely that the \( K_{\text{ATP}} \) channel plays a role in cariporide’s cardioprotective effect.

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REFERENCES


