K\textsubscript{ATP} channel activation reduces the severity of postresuscitation myocardial dysfunction

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Tang, Wanchun, Max Harry Weil, Shijie Sun, Andrej Pernat, and Earl Mason. K\textsubscript{ATP} channel activation reduces the severity of postresuscitation myocardial dysfunction. Am J Physiol Heart Circ Physiol 279: H1609–H1615, 2000.—Postresuscitation myocardial dysfunction has been recognized as a leading cause of the high postresuscitation mortality rate. We investigated the effects of ischemic preconditioning and activation of ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channels on postresuscitation myocardial function. Ventricular fibrillation (VF) was induced in 25 Sprague-Dawley rats. Cardiopulmonary resuscitation (CPR), including mechanical ventilation and precordial compression, was initiated after 4 min of untreated VF. Defibrillation was attempted after 6 min of CPR. The animals were randomized to five groups treated with 1) ischemic preconditioning, 2) K\textsubscript{ATP} channel opener, 3) ischemic preconditioning with K\textsubscript{ATP} channel blocker administered 1 min after VF, 4) K\textsubscript{ATP} channel blocker administered 45 min before induction of ischemic preconditioning, and 5) placebo. Postresuscitation myocardial function, as measured by the rate of left ventricular pressure increase at 40 mmHg, the rate of left ventricular decline, cardiac index, and duration of survival, was significantly improved in both preconditioned and K\textsubscript{ATP} channel opener-treated animals. K\textsubscript{ATP} channel blocker administered 45 min before induction of ischemic preconditioning completely abolished the myocardial protective effects of preconditioning. We conclude that ischemic preconditioning significantly improved post-CPR myocardial function and survival. These results also provide evidence that the myocardial protective effects of ischemic preconditioning are mediated by K\textsubscript{ATP} channel activation.

Ischemic preconditioning was first described by Murry et al. (27) in a canine model of regional myocardial ischemia. These investigators demonstrated that brief periods of myocardial ischemia and reperfusion preceding a sustained ischemic insult were cardioprotective, reducing infarct size to 25% of that seen in a control group. Subsequent studies by other investigators confirmed that ischemic preconditioning decreased infarct size (29), the severity of posts ischemic myocardial contractile dysfunction (26), and the incidence of reperfusion arrhythmias (28). Ischemic preconditioning has been investigated in diverse animal models of regional myocardial ischemia, and these investigations have consistently implicated activation of ATP-sensitive potassium (K\textsubscript{ATP}) channels as the mechanism of ischemic preconditioning (20, 21, 42).

In the present study, we investigated the potential myocardial protective effects of ischemic preconditioning in settings of cardiac arrest and resuscitation. We hypothesized that ischemic preconditioning mitigates postresuscitation myocardial dysfunction, the incidence of postresuscitation ventricular ectopy, and postresuscitation fatal outcomes. We further hypothesized that the myocardial protective effects of ischemic preconditioning during a CPR model of cardiac injury are mediated by activation of K\textsubscript{ATP} channel. Recognizing that the effects of the K\textsubscript{ATP} channel blocker glibenclamide are time dependent in the rat (30, 31), we further investigated the effects of K\textsubscript{ATP} channel blocker on ischemic preconditioning when glibenclamide was administered either before or after ischemic preconditioning.

METHODS

The protocol was approved by the Institutional Animal Care and Use Committee. All animals received humane care in compliance with the Principles of Laboratory Animal Care

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formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Research Council (revised 1996).

Animal preparation. Experiments were performed in an established rat model of cardiac arrest and cardiac resuscitation (36, 39, 41). Twenty-five Sprague-Dawley rats (495–534 g) were fasted overnight, except for free access to water. The animals were anesthetized by an intraperitoneal injection of 45 mg/kg pentobarbital sodium, which was supplemented with additional doses of 10 mg/kg at hourly intervals. No pentobarbital was administered for 30 min before induction of cardiac arrest. The trachea was orally intubated with a 14-gauge cannula (Quick-Cath; Victra Division, Travenol Laboratory, Dallas, TX) mounted on a blunt needle with a 145° angled tip. For measurement of end-tidal PCO₂ (P₄0), an infrared CO₂ analyzer was connected to the tracheal cannula (model 200; Instrumentation Laboratories, Lexington, MA). A Teflon catheter (model UTX022; Becton-Dickinson, Rutherford, NJ) was advanced from the right carotid artery into the left ventricle. This catheter was utilized for the measurement of left ventricular pressure, the rate of left ventricular pressure increase at 40 mmHg (dP/dt₀), and the rate of left ventricular pressure decline (−dP/dt) as previously described (36, 39, 41). An 18-gauge polyethylene catheter (model CPMS-401J-Fa; Cook Critical Care, Bloomington, IN) was advanced through the left external jugular vein into the right atrium. Right atrial pressure was measured with reference to the midchest by using a high-sensitivity transducer (model P-23 9b; Spectramed, Oxnard, CA). A 3-Fr pediatric radial arterial catheter (model C-PUM-301J; Cook Critical Care) was advanced through the right external jugular vein into the right atrium. A precurred guide wire supplied with the catheter was then advanced through the catheter into the right ventricle until a typical right ventricular electrocardiographic injury current confirmed endocardial contact. A second Teflon catheter was advanced through the right femoral artery into the descending thoracic aorta. Blood temperature and cardiac index were measured with this sensor. A third Teflon catheter was advanced through the left femoral vein into the inferior vena cava for sampling blood and for blood transfusion. A conventional limb lead II electrocardiogram was recorded, utilizing subcutaneous needle electrodes.

Experimental procedure. The experimental procedure is diagrammed in Fig. 1. Seventy minutes before VF was induced, animals were randomized into groups treated with ischemic preconditioning, K<sub>ATP</sub> channel opener, ischemic preconditioning with K<sub>ATP</sub> channel blocker administered 45 min before induction of preconditioning (glibenclamide-pre-treated preconditioning), ischemic preconditioning with K<sub>ATP</sub> channel blocker administered 1 min after induction of VF (glibenclamide-posttreated preconditioning), or saline placebo. For the glibenclamide-pretreated preconditioning group, glibenclamide (0.3 mg/kg) was injected into the right atrium immediately after randomization. The dose of glibenclamide was based on earlier studies in a rat model of regional myocardial ischemia by Dr. Gross and associates (30, 31). Ischemic preconditioning was induced by two brief episodes of VF, each of 1-min duration, separated by 10 min of spontaneous circulation. The first episode was induced 25 min, and the second 11 min, before cardiac arrest was induced.

Ventilation was initially established at a tidal volume of 0.65 ml/100 g body wt and a frequency of 100 breaths/min. The tidal volume was then adjusted so as to maintain P<sub>ACO₂</sub> between 35 and 40 mmHg. Fractional inspired O<sub>2</sub> (F<sub>IO₂</sub>) was maintained at 0.21. A progressive increase in 60-Hz current to a maximum of 4 mA was then delivered to the right ventricular endocardium. The current flow was continued for 3 min to preclude spontaneous reversal of VF. Mechanical ventilation was discontinued immediately after the electrocardiogram confirmed VF with mean aortic pressure decreased to <10 mmHg. One minute after the onset of VF, either the K<sub>ATP</sub> channel opener cromakalim (0.5 mg/kg), saline placebo with a total volume of 0.5 ml, or, for animals randomized to the glibenclamide-posttreated preconditioning group, glibenclamide (0.3 mg/kg) was injected into the right atrium. Three minutes later, precordial compression was started and continued for six minutes. A pneumatically driven mechanical chest compressor was utilized as previously described (36, 39). Compression was maintained at a rate of 200 compressions/min with equal compression-relaxation duration. Compression depth was adjusted to reduce the anteroposterior diameter of the chest by 30%. Coincident with the start of precordial compression, mechanical ventilation was resumed. F<sub>O₂</sub> was increased to 1.0. Compression and ventilation were synchronized to maintain two compressions for each ventilation.

Precordial compression was maintained for 6 min, at which time a 2-J direct current (DC) electrical shock was delivered between the anterior chest and the back. If VF was not reversed within 2 s, a second 2-J DC shock was delivered. In unsuccessfully resuscitated animals, precordial compression was restarted and maintained for 30 s before a second sequence of electrical shocks. Animals were monitored for a total of 4 h following successful resuscitation, after which catheters and the endotracheal tube were removed. The animals were then returned to their cages and observed for 48 h. Animals were then killed by intraperitoneal injection of pentobarbital (150 mg/kg). Autopsy was routinely performed to identify injuries to the thoracic and abdominal organs caused by CPR interventions.

Measurements. Aortic pressure, left ventricular and right atrial pressures, electrocardiogram, and P<sub>ACO₂</sub> were continuously recorded on a six-channel recorder (model 2600; Gould, Rolling Meadows, IL) with the use of a personal computer-based data acquisition system supported by CODAS software (version 5; DATAQ Instruments, Akron, OH). The coronary perfusion pressure was calculated as the difference between aortic and time-coincident right atrial pressures in the interval between chest compressions. Myocardial function was assessed from measurements of left ventricular pressure. The frequency response of the sys-

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Fig. 1. Experimental protocol. Pretreat refers to administration of glibenclamide (0.3 mg/kg) for glibenclamide-pretreated preconditioning, and drug refers to administration of cromakalim (0.5 mg/kg), glibenclamide (0.3 mg/kg), or saline placebo. CPR, cardiopulmonary resuscitation; Post res., postresuscitation; MV, mechanical ventilation; VF, ventricular fibrillation; PC, precordial compression; DF, defibrillation.
tem was 35 Hz, with optimal damping. dP/dt40 was measured by analog differentiation as an indicator of isovolemic contractility that is relatively independent of changes in preload and afterload. −dP/dt was measured as an indicator of myocardial relaxation (38, 41, 42).

Statistical analysis. The significance of differences among groups was determined by analysis of variance and Scheffe’s multiple-comparison techniques. Comparisons between time-based measurements within each group were performed with analysis of variance for repeated measurements. The success of resuscitation was analyzed with Fisher’s exact test. Measurements are reported as means ± SD. A value of \( P < 0.05 \) was regarded as significant.

RESULTS

A total of 25 studies were performed and completed. All animals survived animal preparation and were successfully resuscitated in the course of the study. Measurements of baseline myocardial function did not differ significantly among the five groups (see Figs. 3–5).

During CPR, the coronary perfusion pressure was significantly lower in animals treated with cromakalim (Fig. 2). VF voltage in ischemically preconditioned, cromakalim-treated, or glibenclamide-posttreated preconditioned animals was 0.58 ± 0.08, 0.58 ± 0.07, or 0.51 ± 0.07 mV, respectively, 6 min after preconditional compression and was significantly increased compared with VF for placebo controls or glibenclamide-pretreated preconditioned animals (Fig. 2). All animals were successfully resuscitated (Table 1). Three of five preconditioned animals, two of five cromakalim-treated animals, and two of five glibenclamide-posttreated preconditioned animals spontaneously reverted to a supraventricular rhythm and spontaneous circulation. This contrasted with control animals or glibenclamide-pretreated, preconditioned animals, which required multiple electrical shocks before successful defibrillation and return of spontaneous circulation (Table 1). These data suggest that ischemic preconditioning and opening of KATP channels significantly reduce the threshold of defibrillation.

The incidence of postresuscitation ventricular ectopic beats during the first 30 min following successful resuscitation was significantly decreased in animals after ischemic preconditioning, cromakalim treatment, or glibenclamide-posttreated preconditioning (Table 1). Essentially no postresuscitation ventricular ectopic beats appeared in the ischemic preconditioning group. Glibenclamide-posttreated preconditioning did not alter these results (Table 1).

As we previously observed (36, 39, 41), myocardial function, as measured by dP/dt40, −dP/dt, and cardiac index, was significantly decreased in all animals after resuscitation. However, ischemic preconditioning, cromakalim treatment, or glibenclamide-posttreated preconditioning significantly decreased the severity of postresuscitation myocardial dysfunction (Figs. 3–5). Four hours after resuscitation, the dP/dt40 in ischemic preconditioning, cromakalim treatment, or glibenclamide-posttreated preconditioning was 6,675 ± 380, 6,763 ± 410, and 6,665 ± 470 mmHg/s, respectively, and was significantly greater compared with that for control and glibenclamide-pretreated preconditioning (Fig. 3). The similar results were observed in −dP/dt (Fig. 4). As shown in Fig. 5, 4 h after resuscitation, the cardiac index of both cromakalim-treated and ischami-
cally preconditioned animals returned to ~86% of baseline values. The cardiac index in placebo and glibenclamide-pretreated preconditioning was ~52% of baseline values ($P < 0.01$).

All preconditioned, cromakalim-treated, or glibenclamide-posttreated preconditioning-treated animals survived for >48 h. This contrasted with placebo-treated animals, which survived for $23 \pm 3$ h, and glibenclamide-pretreated preconditioning-treated animals, which survived for $22 \pm 2$ h ($P < 0.01$; Table 1). At autopsy, no significant abnormalities were observed on gross examination.

**DISCUSSION**

The present study demonstrated that ischemic preconditioning significantly reduced voltage requirements for successful defibrillation, the incidence of postresuscitation ventricular ectopic beats, the severity of postresuscitation myocardial dysfunction, and postresuscitation fatal outcomes. The $K_{ATP}$ channel opener cromakalim had comparable myocardial protective effects of ischemic preconditioning, except that the incidence of postresuscitation ventricular ectopic beats was slightly lower after ischemic preconditioning. We further confirmed that in the rat model, the $K_{ATP}$ channel blocker glibenclamide had time-dependent effects in the settings of global myocardial ischemia of VF, as it did after regional myocardial ischemia (30, 31).

Postresuscitation myocardial dysfunction has only recently been recognized as a major issue bearing on the high postresuscitation mortality rate. In the Brain Resuscitation Multicenter Clinical Trial I, 262 patients were successfully resuscitated. Approximately 70% of these patients died within the first 72 h. Arterial hypotension, ventricular arrhythmias, and recurrent cardiac arrest were identified as the major causes of death (5). In multicenter clinical studies, ~60% of 407 resuscitated patients died within 72 h. Arterial hypotension and ventricular arrhythmias were identified as predominant causes of death, and only 4.5% were ultimately discharged alive from the hospitals (6). The high incidence and fatal outcomes of postresuscitation myocardial dysfunction prompts our research on options for myocardial preservation during cardiac arrest and resuscitation such that postresuscitation myocardial function and therefore survival may be improved.

Murry et al. (27) first demonstrated that brief episodes of ischemia and reperfusion preceding a sustained ischemic insult markedly reduced the magnitude of myocardial infarction in a dog model of regional myocardial ischemia. This phenomenon, termed "ischemic preconditioning," suggested that myocytes adapted to repetitive ischemic insults and were therefore protected against severe ischemic insults and cell death. This myocardial protective effect was subsequently demonstrated in models of regional myocardial ischemia in pigs (14), rabbits (18), and rats (43). In
each instance, preconditioning significantly reduced infarction size.

It is not yet apparent whether ischemic preconditioning improves myocardial function independently of reducing infarction size. Preconditioning of isolated hearts after either regional (8) or global (1, 7) myocardial ischemia results in greater improvement of left ventricular developed pressure and segment shortening. However, myocardial necrosis also resulted from each of these studies. However, both myocardial contractility and relaxation were significantly improved in preconditioned animals in the rat model reported here. This improved myocardial mechanical function in our model is not likely to be explained by lesser myocardial necrosis. In earlier studies on this rat model with the same severity of ischemic insult, we have consistently failed to identify myocardial necrosis by light microscopy (41). We further observed that postresuscitation ventricular dysrhythmias were essentially eliminated by preconditioning, as in earlier studies in isolated rat hearts (37), intact rats (33), rabbits (8), pigs (35), and, most significantly, human patients (19, 28). It is more likely that preconditioning improves myocardial mechanical function and mitigates postischemic arrhythmias by mechanisms other than infarction reduction.

Current evidence supports the concept that adenosine and its A1 receptor, protein kinase C, and KATP channels are the three major factors that mediate ischemic preconditioning (2, 11). A brief ischemic insult followed by reperfusion generates endogenous adenosine, which in turn activates adenosine receptors. The activated adenosine receptors then couple with G proteins and activate protein kinase C. This process opens KATP channels so that there is an efflux of potassium from cells during repolarization (17, 32). Increases in extracellular potassium reduce the duration of the myocardial action potential and the time available for voltage-dependent calcium influx (32). Increases in intracellular calcium are therefore prevented, increases in myocardial oxygen consumption are minimized, and myocardial ATP concentrations are preserved. The myocytes are therefore better protected during a subsequent ischemic insult. This is consistent with observations in the present study in which approximately one-half of preconditioned or KATP channel opener-treated animals spontaneously reverted to a supraventricular rhythm without the need for electrical defibrillation. Less cytosolic calcium would account for lower defibrillation thresholds (44).

Several very recent studies, however, fail to support the notion that myocardial protective effects of KATP channel activation are explained by shortening of myocardial action potential duration (14, 15). Both Marban and colleagues (16, 23, 24) and Garlid et al. (9) provided evidence that sarcolemmal KATP channels may not account for the myocardial protective effects of ischemic preconditioning. On the basis of experiments in dogs with the mitochondrial KATP channel opener diazoxide and the mitochondrial KATP channel blocker 5-hydroxydecanoic acid (5-HD), these investigators implicated protein kinase C, acting through mitochondrial inner membrane KATP channels, as the mechanism for myocardial protection (16, 23, 24).

Both a nonselective KATP channel opener and blocker were used in the present study. Accordingly, the role of mitochondrial KATP channels was not identified. However, the KATP channel opener cromakalim not only mimicked the effects of ischemic preconditioning on myocardial contractility but also significantly reduced postresuscitation ectopic ventricular dysrhythmias in our study. These findings are reminiscent of other studies on regional myocardial ischemia in which cromakalim significantly reduced the incidence of postischemic ventricular arrhythmias (3). Reperfusion arrhythmias are closely related to changes in the duration of action potential regulated by sarcolemmal KATP channels. The reduction in the incidence of arrhythmias following both preconditioning and cromakalim may therefore be independent of mechanisms that account for infarct size reduction after preconditioning (12). Nevertheless, the mechanisms that account for reduction in ventricular arrhythmias after cromakalim are not yet exposed. Shortening of the action potential duration produced by KATP channel openers is proarrhythmic, yet it may result in inhibition of arrhythmias caused by nonreentrant mechanisms, especially spontaneous and triggered arrhythmias (40).

We also demonstrated in this rat model of cardiac arrest and resuscitation, as previously reported of regional myocardial ischemia by Gross and associates (11), that the effects of the KATP channel blocker glibenclamide are time dependent. It completely abolished the myocardial protective effects of ischemic preconditioning when administered before the induction of preconditioning but had no effect after the onset of cardiac arrest in preconditioned animals. The time delay between the administration of glibenclamide and the initiation of preconditioning may therefore be a critical determinant as to whether this agent blocks preconditioning effectively (30). This particularly applies to the rat model because Grover et al. (13) demonstrated that there is no pharmacological link between adenosine A1 receptors and KATP channels in the ischemic rat heart.

We therefore conclude that ischemic preconditioning significantly decreases the severity of postresuscitation myocardial dysfunction and the incidence of postresuscitation ventricular arrhythmias. Consequently, postresuscitation survival is increased. The pharmacological opening of the KATP channels during cardiac resuscitation mimics the myocardial protective effects of ischemic preconditioning, and this may provide a new option for myocardial preservation during the global myocardial ischemia of cardiac arrest.

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REFERENCES


