δ-Opioid receptor agonist reduces severity of postresuscitation myocardial dysfunction

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Sun, Shijie, Max Harry Weil, Wanchun Tang, Takashi Kamohara, and Kada Klouche. δ-Opioid receptor agonist reduces severity of postresuscitation myocardial dysfunction. Am J Physiol Heart Circ Physiol 287: H969 –H974, 2004. First published March 25, 2004; 10.1152/ajpheart.01171.2003.—Postresuscitation myocardial dysfunction is recognized as a leading cause of death after initially successful cardiopulmonary resuscitation (CPR). In the present study, we hypothesized that a δ-opioid receptor agonist would decrease the severity of postresuscitation myocardial dysfunction and improve survival. Fifteen Sprague-Dawley rats, fasted overnight with access to water, were anesthetized by an injection of 45 mg/kg ip pentobarbital sodium. Additional doses of 10 mg/kg were administered at hourly intervals but not within 30 min before induced ventricular fibrillation (VF). Either the δ-opioid receptor agonist pentazocine (300 μg/kg), pentazocine pretreated with the opioid receptor-blocking agent naloxone (1 mg/kg), or saline placebo was injected into the right atrium after 5 min of untreated VF and 3 min before initiation of CPR. After an additional 8 min of CPR administration, defibrillation was attempted. All animals were successfully resuscitated. Left ventricular rate of pressure increase at 40 mmHg and cardiac index values were significantly improved in pentazocine-treated animals, which also had significantly longer survival times (60 ± 11 vs. 16 ± 7 h; P < 0.01). Except for ease of defibrillation, the beneficial effects of pentazocine were completely abolished by pretreatment with naloxone. The concept of pharmacological hibernation employing a δ-opioid receptor agonist is a novel and promising intervention for minimizing global ischemic injury during CPR and postresuscitation myocardial dysfunction.

Cardiac arrest; cardiopulmonary resuscitation; hibernation; ventricular fibrillation; rat

Although the initial success of cardiopulmonary resuscitation (CPR) is ~39% (range, 13–59%), a majority of victims die within 72 h primarily due to heart failure and/or recurrent ventricular fibrillation (VF). CPR itself therefore yields a functional survival rate of only 1.4–5% (1, 4, 5, 19, 29). Myocardial function is substantially impaired after successful resuscitation from cardiac arrest. We have called this "postresuscitation myocardial dysfunction" (13, 31). These fatal outcomes associated with postresuscitation myocardial dysfunction prompted our search for options by which myocardial injury may be decreased during cardiac arrest and resuscitation (33, 34).

Hibernation is a seasonal state in some animals including black bears and ground squirrels; the metabolic change is triggered by climatic conditions. During hibernation, myocardial O2 consumption (MVO2) and energy production are dramatically reduced. Accordingly, hibernating animals consume <10% of their systemic O2 compared with their prehibernation state (36). Recent evidence indicates that hibernation is triggered by activation of δ-opioid receptors (7, 8, 22). The concept of myocardial hibernation has also been introduced to cardiovascular medicine as a potential therapeutic option for reducing myocardial contractile activity and therefore O2 requirements in settings of regional myocardial ischemia (10). When the magnitudes of myocardial contraction, MVO2, and myocardial substrate utilization were each reduced, myocyte integrity and myocardial viability were preserved (14).

In preliminary studies on pigs, we demonstrated that pharmacological activation of δ-opioid receptors significantly reduced MVO2 during the global myocardial ischemia of cardiac arrest (35). In the present study on an established rat model of CPR, we investigated the potential myocardial protective effects of activation of δ-opioid receptors. We hypothesized that activation of δ-opioid receptors mitigates postresuscitation myocardial dysfunction and increases the duration of postresuscitation survival.

MATERIALS AND METHODS

This protocol was approved by the Institutional Animal Care and Use Committee of the Institute of Critical Care Medicine. All animals received humane care in compliance with the Principles of Laboratory Animal Care 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 Institutes of Health (NIH publication 0-309-05337-3, Revised 1996). The Institute of Critical Care Medicine is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Animal preparation. Fifteen experiments were performed and completed. Briefly, 15 Sprague-Dawley rats (body wt, 498–554 g) were fasted overnight but received free access to water. The animals were anesthetized by an injection of 45 mg/kg ip pentobarbital sodium. Additional doses of 10 mg/kg were administered at hourly intervals but not within 30 min preceding the onset of cardiac arrest. The trachea was orally intubated with a 14-gauge cannula mounted on a blunt needle with a 145° angled tip (Abbocath-T, Abbott Hospital; North Chicago, IL) as previously described (30, 32, 33).

End-tidal P CO2 (PETCO2) was measured with a side-stream infrared CO2 analyzer (model 200, Instrumentation Laboratories; Lexington, MA) interfaced between the tracheal cannula and the respirator. For measurement of left ventricular (LV) pressure, the rate of LV pressure increase at 40 mmHg (dP/dt0), and the rate of LV pressure decline (–dP/dt), a 23-gauge polyethylene (PE) catheter (Intramedic PE-50, Becton-Dickinson; Sparks, MD) was advanced from the right carotid...
artery into the left ventricle. A 23-gauge PE catheter (PE-50, Becton-Dickinson) was advanced through the left external jugular vein and the superior vena cava into the right atrium. Right atrial pressure was measured with reference to the midchest with a high-sensitivity pressure transducer (model 42584-01, Abbott Critical Care Systems; North Chicago, IL). A 4-F PE catheter (model C-PMS-401J, Cook Critical Care; Bloomington, IN) was advanced through the right external jugular vein into the right atrium. A precured guide wire supplied with the catheter was then advanced through the catheter into the right ventricle until an endocardial electrogram was confirmed. A PE-90 catheter (Becton-Dickinson) was advanced through the left femoral artery into the thoracic aorta for measurement of aortic pressure with the high-sensitivity pressure transducer. A thermocouple microprobe 10 cm in length and 0.5 mm in diameter (9030-12-D-34, Columbus Instruments; Columbus, OH) was advanced from the right femoral artery into the descending thoracic aorta. Blood temperature was measured with this sensor. A PE-50 catheter (Becton-Dickinson) was advanced through the left femoral vein into the inferior vena cava for sampling venous blood and for blood transfusion. ECG lead II was continuously recorded. A heat lamp was used to maintain body temperature at 36.8°C (±0.2%) throughout the experiment.

Experimental procedure. Mechanical ventilation was initially established at a tidal volume of 0.65 ml/100 g of body wt and a frequency of 100 breaths/min. The tidal volume was then adjusted to maintain PET CO 2 between 35 and 40 mmHg. The inspired O 2 fraction (F I O 2 ) was maintained at 0.21. For animals randomized to receive opioid receptor blocking agent with pentazocine, naloxone in a dose of 1 mg/kg was injected 15 min before induction of VF. 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 after onset of VF. Either pentazocine in a dose of 300 μg/kg or saline placebo was administered after 5 min of untreated VF. Precordial compression was then begun 8 min after the onset of VF with a pneumatically driven mechanical chest compressor (Dickinson) was advanced through the left external jugular vein and the superior vena cava into the right atrium. Right atrial pressure was measured with reference to the midchest with a high-sensitivity pressure transducer. A thermocouple microprobe 10 cm in length and 0.5 mm in diameter (9030-12-D-34, Columbus Instruments; Columbus, OH) was advanced from the right femoral artery into the descending thoracic aorta. Blood temperature was measured with this sensor. A PE-50 catheter (Becton-Dickinson) was advanced through the left femoral vein into the inferior vena cava for sampling venous blood and for blood transfusion. ECG lead II was continuously recorded. A heat lamp was used to maintain body temperature at 36.8°C (±0.2%) throughout the experiment.

Table 1. Effects of treatment on number of defibrillations and duration of survival

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Shocks</th>
<th>Urine Volume, ml</th>
<th>Survival, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentazocine</td>
<td>0.6±0.5*</td>
<td>2.2±0.841</td>
<td>60±111</td>
</tr>
<tr>
<td>Naloxone + pentazocine</td>
<td>0.4±0.5*</td>
<td>1.1±0.65*</td>
<td>15±7</td>
</tr>
<tr>
<td>Control</td>
<td>3.6±2.6</td>
<td>0.4±0.37</td>
<td>16±7</td>
</tr>
</tbody>
</table>

Values are means ± SD. Urine volume, total urine volume collected during first 4 h after resuscitation. *P < 0.05; †P < 0.01 vs. control.
saline placebo-treated and naloxine-pretreated animals (104 ± 28 vs. 139 ± 22 vs. 155 ± 15 mmHg, respectively; P < 0.05).

These changes support the hypothesis that animals treated with an opioid receptor agonist had less O₂ extraction during CPR. There was no significant difference in arterial lactate level during chest compression between the three groups. However, the arterial lactate level was significantly lower in pentazocine-treated animals during the postresuscitation phase (Fig. 1). Because there was significantly better myocardial function after resuscitation in animals treated with pentazocine, this decreased level of lactate after resuscitation may also indicate a favorable systemic metabolic effect after pentazocine administration.

As we previously observed, myocardial function as measured by dP/dt₄₀, −dP/dt, and CI was significantly decreased in all animals after resuscitation (31, 33). However, the severity of postresuscitation myocardial dysfunction was significantly less in pentazocine-treated animals when compared with saline placebo-treated animals (Figs. 2–4). As shown in Fig. 4, the CI of pentazocine-treated animals returned to 74% of baseline values at 4 h after restoration of spontaneous circulation, whereas the CI in placebo-treated animals was only 44% of baseline values (P < 0.01). These effects of pentazocine, however, were completely abolished by pretreatment with naloxone (Figs. 2–4).

All pentazocine-treated animals survived for >48 h. This contrasted with placebo-treated animals, which survived for only 16 ± 7 h (P < 0.01; see Table 1). This beneficial effect of pentazocine was again abolished by pretreatment with naloxone. No gross abnormalities were observed at autopsy in any study groups.

DISCUSSION

The present study demonstrated that the nonselective δ-opioid receptor agonist pentazocine strikingly reduced the severity
of postresuscitation myocardial dysfunction. The number of electrical shocks required for defibrillation was significantly reduced. This was associated with a significantly prolonged postresuscitation duration of survival. Significantly smaller arteriovenous $O_2$ differences during CPR were observed in pentazocine-treated animals. However, there were no differences in CPP values and $P_{T\text{CO}_2}$ concentrations between the three groups. Based on earlier documentation of the high correlation between CPP and $P_{T\text{CO}_2}$ with the forward flow generated by precordial compression (12, 17), we found additional support that total body $O_2$ consumption was reduced. Lower arterial lactate concentrations after successful resuscitation supported the hypothesis that animals treated with pentazocine had significantly improved postresuscitation myocardial function. Interestingly, the nonselective opioid receptor blocking agent naloxone abolished all beneficial effects of pentazocine except the reduced number of electrical shocks. The effect of pentazocine on the number of electrical shocks therefore occurs through a mechanism that is unrelated to opioid receptors.

Postresuscitation myocardial dysfunction has recently been recognized as one of the leading causes of the high postresuscitation mortality rate. In settings of out-of-hospital cardiac arrest, ~40% of the victims of sudden death were initially resuscitated. However, 70% of these patients died within the first week. Refractory arterial hypotension, ventricular arrhythmias, and recurrent VF were identified as the major causes of these fatal outcomes (4, 5). Our most recent study further demonstrated that the severity of postresuscitation myocardial mechanical dysfunction is closely related with blood troponin I levels (23). The high incidence and fatal outcome of postresuscitation myocardial dysfunction prompted our continuing search for options by which myocardial function would be preserved during cardiac arrest and resuscitation such that survival may be improved. The present study in which untreated (placebo) animals or naloxone-pretreated animals had significantly more severe postresuscitation myocardial dysfunction and survived for <16 h after initially successful resuscitation pinpointed the potential value of treatment with a $\delta$-opioid agonist, after which myocardial function and survival were very significantly improved ($P < 0.01$).

The concept of myocardial hibernation has previously entered clinical medicine. In 1978, Diamond et al. (10) described an adaptive reduction of energy utilization in settings of coronary artery disease with reduced physical activity and substrate requirements as hibernation. These authors pointed to adaptive reduction of myocardial contractile activity and therefore $O_2$ requirements when coronary blood flow was occluded. When the magnitude of myocardial contraction, $MVO_2$, and myocardial substrate utilization were decreased, myocardial viability and cellular integrity were preserved (14).

Rahimtoola (24, 25) applied similar hypotheses to patients with chronic LV dysfunction due to coronary artery disease. Patients completely recovered myocardial function after revascularization with coronary aortic bypass operations. Rahimtoola characterized “hibernating myocardium” as either or both an acute or chronic state of myocardial ischemia. Although myocardial contractility was impaired, a new equilibrium between energy supply and energy demand preserved survival of the affected myocardium.

During global myocardial ischemia after cardiac arrest, fatty acid oxidation ceases. Cardiac function is then dependent on anaerobic glycolysis. This emergency pathway provides only ~5% of the energy that is normally produced by oxidation of glucose. Consequently, the energy requirements of the fibrillating heart rapidly exceed availability (6). All residual ATP is hydrolyzed, and anaerobically generated lactate accounts for excesses of $H^+$ (15, 16). The excesses of $H^+$ are buffered by intracellular bicarbonate such that myocardial $PCO_2$ is strikingly increased. In initial studies at our institute on myocardial tissue $PCO_2$, the $PCO_2$ increased from 45 to $>400$ mmHg, and coronary venous lactate increased from 0.8 to $>12$ mmol/l during 7 min of untreated VF (18, 20). Our hypothesis postulates that pharmacological interventions that result in rapid reductions of myocardial metabolism would minimize ischemic injury and prevent or minimize postresuscitation myocardial dysfunction.

Three unique classes of opioid receptors have been identified in the central nervous system; these are designated $\mu$, $\kappa$, and $\delta$. Moreover, subtypes have more recently been identified for each class of receptor. Each receptor group has a discrete pharmacological profile (9). Schultz and colleagues (26, 27) first demonstrated that activation of opioid receptors, especially $\delta$-opioid receptors, plays an important role in the myocardial protective effects of ischemic preconditioning. More recent studies indicate that hibernation appears to be mediated predominantly by the $\delta$-class of receptors, and more specifically, the $\delta_1$- and $\delta_2$-receptors (2, 21). In a recent study on isolated, perfused rabbit hearts, Benedict et al. (2) administered opiates as a pharmacological option akin to that of myocardial hibernation. $MVO_2$ was reduced in hearts pretreated with the $\delta$-opioid agonists bepareorphine and pentazocine. Postischemic myocardial mechanical function was significantly improved. Histologically, the hearts treated with the $\delta$-opioid agonists maintained ultrastructural integrity when compared with untreated controls (2). Our results are consistent with these in vitro studies, which support the concept that myocardial hibernation is mediated by the $\delta$-opioid receptors. However, a most recent study by Wu et al. (37) demonstrated that the selective $\mu$-opioid receptor agonist [Dmt(1)]DALDA protected the isolated guinea pig heart against ischemia-induced myocardial stunning by a mechanism that is unrelated to opioid receptors. Because there are no $\mu$-opioid receptors expressed in the adult rat myocardium (27), the myocardial protective effects of pentazocine likely result from activation of $\delta$-opioid receptors in the present study.

The distinctive roles of $\delta_1$- and $\delta_2$-opioid receptor activation in myocardial protection have recently been investigated. In a rat model of regional myocardial ischemia, Fryer et al. (11) demonstrated that after 30 min of coronary artery occlusion and 2 h of reperfusion, the infarct size was significantly reduced from 60 to 37% in animals treated with the $\delta$-opioid receptor agonist DADLE. Interestingly, this protective effect was completely abolished by the selective $\delta_1$- but not the $\delta_2$-opioid receptor antagonist. In both rat and rabbit isolated heart preparations, Schultz et al. (28) and Bolling et al. (3) independently demonstrated that activation of the $\delta_1$-opioid receptor reduced infarct size but did not improve postsischemic myocardial function. However, a selective $\delta_2$-antagonist completely abolished the improved postsischemic myocardial mechanical function that followed...
opioid activation. These studies support the concept that the protective effects of δ₁-receptors operate predominantly during the ischemic phase and δ₂-agonists operate predominantly during the reperfusion phase. Because a nonselective δ-opioid receptor agonist and antagonist were employed in the present study, the individual myocardial protective roles of the subtype opioid receptors remain unknown in the setting of the global myocardial ischemia of cardiac arrest and resuscitation.

We acknowledge the limitations of the present study as follows: 1) because of the small size of rat hearts and the rapid heart rates, it is difficult to induce and maintain VF in this model. The effects of pentazocine on reduced numbers of electrical shocks may not apply to large-animal models or humans. 2) Although all pentazocine-treated animals expressed normal neurological function (eating, drinking, and walking) 24 h after resuscitation, the neurological outcomes were not compared between the three groups because nearly all of the animals died within 24 h after resuscitation in both saline- and naloxone-treated groups. 3) Although the depth of anesthesia and the body temperatures were maintained consistently in all animals, we acknowledge that because pentazocine itself is an anesthetic agent, we cannot exclude the possibility that some of its myocardial protective mechanism may be from an increased depth of anesthesia. As a result, myocardial temperature and therefore metabolism would be reduced.

We conclude that the δ-opioid receptor agonist decreases myocardial ischemic injury, facilitates restoration of spontaneous circulation, and reduces postresuscitation myocardial dysfunction. These improvements were associated with highly significant increases in postresuscitation survival. Pharmacological hibernation may therefore represent a promising new option for myocardial protection during cardiac arrest and resuscitation.

GRANTS

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REFERENCES


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