Opiate Drugs and \( \delta \)-Receptor–Mediated Myocardial Protection

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Background—Hypothermic myocardial arrest is necessary to complete most cardiac surgery, which limits the success of such operations. Similarly, cold, inhospitable environments limit the survival of warm-blooded animals. Animals have successfully adapted to this challenge through hibernation. Hibernation is an energy-conserving state, now known to be governed by cyclical variation in endogenous opiate compounds. It may also be induced in nonhibernators via hibernating animal serum factors or \( \delta \)-opiate peptides. Furthermore, hibernation-induction triggers extend organ preservation in many models. This study examined whether opiate drugs with an affinity for the \( \delta \)-opiate receptor confer similar protection.

Methods and Results—Isolated hearts harvested from New Zealand White rabbits were treated with either cardioplegia alone or \( \delta \)-opiate drugs (fentanyl, morphine, buprenorphine, pentazocine) followed by 2 hours of 34°C ischemia. Hearts were then reperfused, and functional and metabolic indices of treated groups were compared with untreated controls. Isovolumic developed pressure, coronary flow, and oxygen consumption were compared as a percent of preischemia versus 45 minutes after reflow. Developed pressure and oxygen consumption were better preserved in the morphine, buprenorphine, and pentazocine groups when compared with cardioplegia alone.

Conclusions—Drugs with \( \delta \)-opiate activity confer myocardial protection, which is additive to cardioplegia. Use of \( \delta \)-opiate drugs in this context may have important clinical implications. (Circulation. 1999;100[suppl II]:II-357–II-360.)

Key Words: cardioplegia ■ cardiopulmonary bypass ■ drugs ■ hibernation ■ receptors

Despite years of experience, clinicians are still challenged by the perioperative care of the cardiac patient. Most operations on the heart require the use of hypothermic, ischemic cardiac arrest, which limits the success of such operations. Similarly, warm-blooded animals are challenged by life in cold, inhospitable environments. Many animals have successfully adapted to this challenge via hibernation. A fascinating concept is that the biological mechanism of hibernation may be duplicated in humans, thereby inducing a profound state of energy conservation throughout the body or at the organ level. Conceivable applications for “induced hibernation” include transplantation and cardiac surgery.

Hibernation only occurs in certain animals, such as black bears, woodchucks, and ground squirrels, in response to climatic conditions. During this process, the metabolic processes of the body are dramatically slowed down. In fact, hibernating animals use only \( \approx \)10% of their normal, active energy expenditure. Hibernation is a process mediated by cyclical variation in endogenous opiate compounds. It is also evident that hibernation is not only opiate-mediated, but that the \( \delta \)-opiate receptor in particular is responsible. Additionally, serum from hibernating animals, when injected into summer-active animals, induces hibernation behavior and physiology. Conversely, hibernation can be reversed by opiate antagonists.

Given that hibernation is a state of energy conservation and is reproducible with the administration of \( \delta \)-opiates, potential implications for organ preservation arise. In fact, using hibernation triggers to extend organ viability has been done successfully in many models, including myocardial protection. Coincidentally, nonpeptide, opiate drugs are the mainstay of cardiac anesthetics. These drugs are used in this setting for their potent analgesic properties and cardiovascular stability. To some degree, each opiate drug in common use is thought to possess an affinity for the \( \delta \)-opiate receptor. The hypothesis of the current investigation was that opiate drugs with activity at the \( \delta \)-receptor will confer protection against ischemic insult in a fashion similar to endogenous hibernation-induction triggers and the synthetic \( \delta \)-peptides.

Methods

The model used in this study was an isolated, beating rabbit heart. Briefly, New Zealand White rabbits (male or female; body weight, 2.0 to 2.5 kg) were anesthetized with sodium pentobarbital (45 mg/kg IV) and heparinized (700 U/kg IV). Each heart was excised, and the aorta was immediately cannulated in the Langendorff mode. The heart was then perfused with a physiological salt solution (pH 7.4) containing (in mmol/L): NaCl 118, KCl 4.0, NaHCO\(_3\) 22.3, glucose 11.1, KH\(_2\)PO\(_4\) 0.66, MgCl\(_2\) 1.23, and CaCl\(_2\) 2.38. The solution was equilibrated with 95% O\(_2\)/5% CO\(_2\) at 37°C and passed...
twice through filters with a pore size of 3.0 μm. Perfusion pressure was maintained at 90 mm Hg.

A fluid-filled latex balloon was inserted into the left ventricle through a small incision in the left atrium. The balloon was connected to a pressure transducer for continuous measurement of left ventricular pressure, and its first derivative, dP/dT. Vena cavae and ayzygous veins were ligated. A cannula was placed in the pulmonary artery to collect measurements of coronary flow (CF) and oxygen content. Analog signals from the above measurements were digitized to an online computer (AST Premium 386, AST Research Inc). Left ventricular function was characterized as developed pressure, which was defined as peak systolic pressure minus end-diastolic pressure. Myocardial oxygen consumption (MV˙O2) was calculated using the following formula: MV˙O2 = CF(cc · min⁻¹ · g⁻¹) × Bunsen solubility coefficient of O₂ at 37°C (22.7 μL of O₂ · atm⁻¹ · cc of perfusate /760). The partial pressure of O₂ of the perfusate was 665 mm Hg. CF was measured by collecting the coronary effluent in a graduated cylinder. Oxygen extraction was calculated as MV˙O2 divided by O₂ content of the perfusate. Optimal left ventricular balloon volume was determined after instrumentation was complete to ensure that pressure measurements after interventions were within the appropriate compliance range for each experiment. The volume was adjusted such that developed pressure ranged from 100 to 140 mm Hg. This volume was then maintained during baseline and reperfusion conditions. Baseline data were recorded after a 30-minute stabilization and equilibration period. All isolated hearts were maintained in a 37°C water-jacketed organ bath during the entire baseline period. At the conclusion of the baseline period, the physiological salt solution infusion was stopped, and 60 cc of 4°C cardioplegia solution was injected into the aorta over 1 minute to commence the 2-hour 34°C period of ischemia. In the treatment groups, the test drug was infused over 5 minutes at the end of the baseline equilibration period at a concentration thought to be clinically relevant (based on current human dosing). These concentrations were as follows: morphine 1 μg/cc, fentanyl 100 ng/cc, buprenorphine 500 ng/cc, and pentazocine 5 μg/cc. They were also equipotent. A total of 20 cc of each concentration was added to the physiological salt perfusate and delivered over 5 minutes. Each heart was allowed to re-equilibrate for 5 minutes before standard cardioplegia-induced ischemia. These hearts were then treated in a similar fashion to controls. At the conclusion of each experiment, each heart was harvested for histological examination and grading, which was accomplished via a semiquantitative visual scale.

Statistical analysis was performed using the Stat-View program (Abacus Concepts Inc). Data were evaluated using ANOVA (Scheffe’s test). Differences between groups were considered significant at P < 0.05. All animal treatments were performed in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research. All work was performed in our laboratory demonstrated the efficacy of natural hibernation induction triggers and the synthetic compound D-Ala2-Leu5-enkephalin (DADLE) in mediating myocardial protection that is superior to cardioplegia. The synthetic peptide D-phen2,5-enkephalin (DPDPE), a δ-opiate, failed elicit such protection. The objective of the present investigation was to bring our previous work, using synthetic peptides and animal serum, 1 step closer to the clinical realm by using opiates in common clinical use. Although data exist indicating that some of these commonly used drugs are active and metabolic integrity after 2 hours of ischemia. Control hearts developed only 38% of their original contractility on reperfusion. Opiate drugs used for pretreatment in this study were associated with varying degrees of functional improvement after global myocardial ischemia. Fentanyl, which is generally regarded as a potent μ-receptor agonist, offered no significant improvement in functional recovery. Morphine pretreatment was associated with preserved functional recovery, which implies activity at the δ-receptor in addition to its known μ-affinity. Buprenorphine and pentazocine were chosen as treatment drugs because of their theoretically higher affinity for δ-opiate receptors. Pretreatment with these compounds resulted in an impressive degree of myocardial protection against ischemic insult. On histological examination, the functionally improved groups also showed preserved ultrastructural integrity (intact mitochondrial architecture and glycogen storage granules and less free intracellular calcium) when compared with controls.

**Results**

In the preischemia interval, no significant differences existed between groups in any of the functional parameters recorded. The Table summarizes the outcome of the different treatment groups in the postischemia interval. Functional recovery was compared with untreated controls. Isovolumic developed pressure, CF, and MV˙O2 were compared as a percent of preischemia versus 45 minutes after reflow. Data are mean ± SEM.

The functional recovery of each treatment group is expressed as the percent of developed pressure after reperfusion compared with the preischemic value. Metabolic recovery was assessed in a similar fashion using MV˙O2. Isolated hearts in this study showed a marked decrement in both functional and metabolic integrity after 2 hours of ischemia. Control hearts developed only 38% of their original contractility on reperfusion. Opiate drugs used for pretreatment in this study were associated with varying degrees of functional improvement after global myocardial ischemia. Fentanyl, which is generally regarded as a potent μ-receptor agonist, offered no significant improvement in functional recovery. Morphine pretreatment was associated with preserved functional recovery, which implies activity at the δ-receptor in addition to its known μ-affinity. Buprenorphine and pentazocine were chosen as treatment drugs because of their theoretically higher affinity for δ-opiate receptors. Pretreatment with these compounds resulted in an impressive degree of myocardial protection against ischemic insult. On histological examination, the functionally improved groups also showed preserved ultrastructural integrity (intact mitochondrial architecture and glycogen storage granules and less free intracellular calcium) when compared with controls.

**Discussion**

Previous investigations demonstrated that animal hibernation is a process due, in large part, to circannual variation of opiate-like compounds in endogenous serum. Specifically, the yet-to-be-identified endogenous δ-opiate agonist, heretofore known as HIT (hibernation-induction trigger), may be the responsible serum factor. Synthetic compounds that resemble HIT in their affinity for this receptor have also demonstrated efficacy in eliciting hibernation behavior and physiology. Improved energy conservation and organ preservation have been attributed to pretreatment with both HIT and synthetic analogs. Specifically, hearts exposed to global ischemia seem to be relatively protected from postischemic dysfunction compared with those not receiving preischemic conditioning via δ-opiate compounds.

We think that the δ-opiate receptor elicits cardioprotective effects and, more specifically, that the δ-receptor is responsible for the protection observed in our studies. Previous work performed in our laboratory demonstrated the efficacy of natural hibernation induction triggers and the synthetic compound D-Ala2-Leu5-enkephalin (DADLE) in mediating myocardial protection that is superior to cardioplegia. The synthetic peptide D-phen2,5-enkephalin (DPDPE), a δ-opiate, failed elicit such protection. The objective of the present investigation was to bring our previous work, using synthetic peptides and animal serum, 1 step closer to the clinical realm by using opiates in common clinical use. Although data exist indicating that some of these commonly used drugs are active and metabolic integrity after 2 hours of ischemia. Control hearts developed only 38% of their original contractility on reperfusion. Opiate drugs used for pretreatment in this study were associated with varying degrees of functional improvement after global myocardial ischemia. Fentanyl, which is generally regarded as a potent μ-receptor agonist, offered no significant improvement in functional recovery. Morphine pretreatment was associated with preserved functional recovery, which implies activity at the δ-receptor in addition to its known μ-affinity. Buprenorphine and pentazocine were chosen as treatment drugs because of their theoretically higher affinity for δ-opiate receptors. Pretreatment with these compounds resulted in an impressive degree of myocardial protection against ischemic insult. On histological examination, the functionally improved groups also showed preserved ultrastructural integrity (intact mitochondrial architecture and glycogen storage granules and less free intracellular calcium) when compared with controls.
at the δ-receptor,15,16,21,22 no data exist regarding their respective affinities for δ-subtypes. We included fentanyl in the study as a contrast to the δ-compounds because of its known strong μ-receptor affinity.15 Finally, various investigations showed that morphine has an affinity at both μ- and δ-receptors.21 These previous studies indicate the binding affinities of the drugs used in this study for opiate receptor subtypes, and we chose the drugs on the basis of these data.

Teleologically, the concept of energy conservation, at the whole body and organ level, makes sense for the mammalian species using it. Known hibernators, such as squirrels, bears, hedgehogs and woodchucks, use this process to conserve much of their energy at a time when temperatures are subfreezing and food is scarce.23 Profound behavioral and metabolic changes are hallmarks of this process, which includes hypothermia, bradycardia, respiratory depression, hypophagia, and behavioral changes resembling anesthesia or deep sleep.4 These changes allow prolonged survival despite the presence of extreme cold and the absence of nutritional intake for months at a time.

Dawe and Spurrier24 were the first to demonstrate an endogenous “trigger” responsible for hibernation. They showed that plasma from the hibernating ground squirrel, when injected in either summer-active ground squirrels or woodchucks, produced a behavioral and physiological state resembling hibernation. The identity of the endogenous hibernation trigger has been elusive, however. Recent evidence implicates a plasma protein that is thermolabile, protease sensitive, and nuclease insensitive.2 HIT seems to be albumin-related, and its manifestations in hibernating species may be dependent on cyclical variations in plasma albumin concentrations.25

Oeltgen et al26 were the first investigators to successfully simulate hibernation in a nonhibernating organ model. They showed an increase in transplant graft survival time when using HIT to cause energy conservation in ischemic dog organs. When canine donor lungs were preconditioned with serum from hibernating woodchucks, a remarkable improvement in graft survival time was demonstrated. The synthetic compound DADLE was at least as effective as HIT in the same organ preparation.6 In separate studies, Bolling et al12,13 used both the natural HIT and the synthetic opiate DADLE to create significant myocardial protection in an isolated rabbit heart model. Further studies using receptor antagonists will provide more compelling evidence that drug- and serum-induced cardioprotection is, in fact, due to opioid receptor activation. Currently, work is in progress in our laboratory using the δ-subtype specific antagonists naltrindole and naltrindole and the nonspecific opiate antagonists naltrexone and naltrexone to provide such evidence.

In light of these recent discoveries, it is interesting and perhaps fortuitous that opiates are the primary component of cardiac anesthetics. Their popularity in this context stems from their potent analgesic effects combined with a high degree of hemodynamic stability. Given the fact that δ-opiates govern hibernation-related energy conservation and that most opiate drugs possess some δ-activity, their usefulness may extend beyond the provision of patient comfort and stability during cardiac operations. The drugs used in this study were chosen because they are commonly used in humans and are thus clinically relevant. Hence, this work reflects an effort to bring the concept of opiate-mediated energy conservation closer to clinical human application.

Although it is conceivable that opiates may someday comprise a portion of standard cardioplegic regimens, their use is not without potential pitfalls. Side effects of the opiate drugs are well known, and they include respiratory depression, nausea, pruritus, and sedation. These would need to be taken into account if the drugs were given with the specific goal of myocardial protection. It seems, however, that the concentration necessary to achieve protective effects is well below that achieved using standard intravenous analgesic regimens. Morphine is a part of many cardiac premedication regimens, and its use may pose a problem when present in concert with δ-opiate agents used specifically for myocardial protection. As previous studies have shown, morphine may antagonize δ-mediated effects because it is an opiate μ-agonist,6 thus negating the beneficial effects of δ-agents. However, this study and others26 indicate that morphine itself has activity at the δ-receptor, which implies beneficial effects with respect to myocardial protection. Finally, the use of agonist/antagonist drugs to achieve myocardial protection could antagonize the analgesic effects of μ-active agents like morphine and fentanyl. This would need to be taken into account before using opiates for such purposes.

Although both HIT and synthetic δ-peptides can mimic hibernation physiology in animal models, neither compound is presently applicable for human administration. Therefore, we attempted to simulate this hibernation-like energy conservation by using common drugs that possess δ-opiate activity. The exact mechanism of δ-mediated energy conservation has yet to be elucidated, but recent investigation points to several hypothetical pathways. Evidence exists that preservation of intracellular ATP, through inhibition of adenosine triphosphatases, may improve ischemic tolerance.27 These agents may also protect myocyte membranes against oxygen-derived free radicals or improve the release of calcium from the sarcoplasmatic reticulum.28–31 Altered DNA and protein synthesis are also associated with hibernation induction,32 and this may play a part in the profound metabolic inhibition associated with δ-opioid activity. Opioids may also inhibit stimulatory G-proteins33 or affect energy conservation through a potassium-ATP mechanism.26 At present, research continues in an effort to identify and characterize HIT and elucidate its exact mechanism of action. Specifically, we are examining the effects of glyburide, a known potassium-ATP antagonist, and pertussis toxin, a G protein antagonist, and their interaction with opiate-related phenomena.

The present study demonstrated that pretreatment with commonly used medications that have δ-opioid activity improved posts ischemic metabolism and function in an isolated rabbit heart model. Administration of the compounds before global ischemia, which is followed by improvement in posts ischemic recovery, is consistent with opiate receptor activation, as demonstrated in the aforementioned studies using HIT and peptide opiates. The enhancement of myocardial preservation afforded by these compounds is similar in magnitude to that of HIT and the synthetic opiate DADLE.
This surgically analogous investigation was not intended to create an infarct model. Rather, our goal was to mimic the global stunning attributable to the use of cardioplegia and ischemic arrest that is necessary to perform cardiac surgery in humans. This work indicates that the compounds morphine, buprenorphine, and pentazocine have an affinity for the δ-opiate receptor and, thus, may have clinical utility in ischemic organ preservation.

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