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Opioids confer myocardial tolerance to ischemia: Interaction of delta opioid agonists and antagonists

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Background: Mammalian hibernation biology is now known to be mediated by delta opioids. The altered myocellular physiology of hibernation closely parallels that of hypothermic ischemia used to protect the heart for cardiac surgery.

Methods and Results: The present study examined the interaction of delta opioid agonists and antagonists on myocardial tolerance to ischemia. By means of a nonhibernating isolated rabbit heart model, functional and metabolic myocardial parameters were assessed during nonischemic baseline and postischemic recovery periods. Control hearts with standard cardioplegic protection alone were compared with those with cardioplegia plus preperfusion with a delta opioid agonist, a delta opioid antagonist, or both. All hearts were then subjected to 2 hours of global ischemia. Compared with cardioplegia alone, postischemic left ventricular developed pressure, coronary flows, and myocardial oxygen consumption were all increased with administration of delta opioid agonists and decreased below baseline with delta opioid antagonists. Functional recovery of left ventricular developed pressure was improved with opioids (control hearts: 36 ± 3 mm Hg vs hearts with cardioplegia plus delta opioid agonist: 65 ± 5 mm Hg, $P < .01$) and inhibited with antagonists (control hearts: 36 ± 3 mm Hg vs hearts with cardioplegia plus delta opioid antagonist: 17 ± 5 mm Hg, $P < .05$), and true to form, the protective opioid effect was negated when combined with an antagonist (control hearts: 36 ± 3 mm Hg vs hearts with cardioplegia plus delta opioid agonist and delta opioid antagonist: 42 ± 4 mm Hg, P = not significant).

Conclusions: This study demonstrates that cardiac tolerance to ischemia may be mediated by delta opioids.

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Presently, cardioplegia and hypothermia provide considerable myocardial protection during the induced ischemia of cardiac surgery. However, poor myocardial tolerance to ischemia, as seen with perioperative ventricular dysfunction and stunning, remain significant clinical problems. Interestingly, the subcellular and molecular myocytic changes seen during mammalian hibernation closely parallel the altered myocyte physiology seen with hypothermic cardioplegic arrest. Both undergo depletion of energy stores, intracellular acidosis, hypoxia, hypothermia, and cellular volume shifts. Yet the myocardium of the hibernating mammal can endure these alterations resiliently for months, whereas the duration of induced ischemia tolerated surgically is limited to hours. Through its effective reversal by opiate antagonists, an opiate nature to this hibernation-mediated ischemic tolerance has been established. Physical chemistry analysis indicates that these peptides are similar to delta opioids,

TABLE 1. Postischemic functional and metabolic indices

	n	LVDP (mm Hg)	CF (mL/min)	MVO_2 ($\mu L\ O_2$/min)	+dP/dt (mm Hg/s)	−dP/dt (mm Hg/s)
CON	10	36 ± 3	60 ± 4	45 ± 3	37 ± 3	33 ± 2
DPDPE	4	34 ± 4	76 ± 5	71 ± 7*	43 ± 7	38 ± 6
DADLE	7	65 ± 5*	80 ± 5	73 ± 5*	69 ± 7*	66 ± 6*
Nx 2×10^{-4}	4	17 ± 5*	47 ± 19	37 ± 15	27 ± 18	27 ± 16
Nx 2×10^{-5}	4	27 ± 8	80 ± 15	58 ± 10	27 ± 9	26 ± 8
Nx 2×10^{-6}	4	17 ± 6*	57 ± 8	46 ± 5	19 ± 6	16 ± 5
Nx 2×10^{-5} + DADLE	4	42 ± 4	72 ± 14	63 ± 10	45 ± 6	38 ± 4
Nx 2×10^{-6} + DADLE	4	26 ± 4	64 ± 5	58 ± 4	30 ± 5	27 ± 4
Nx 2×10^{-7} + DADLE	5	45 ± 4	60 ± 7	65 ± 6*	49 ± 5	43 ± 5
Nx 2×10^{-9} + DADLE	4	44 ± 3	87 ± 12*	79 ± 9*	50 ± 2	42 ± 2
BNTX	4	53 ± 9*	88 ± 5*	78 ± 5*	55 ± 10	47 ± 9
NTB	4	23 ± 5	76 ± 18	58 ± 11	24 ± 6	23 ± 7

Data are expressed as means ± SD. Preischemia solution administration: CON, standard cardioplegia; Nx, naltrexone delta 1 and 2 antagonist expressed in molar concentrations. Postischemia functional parameters: CF, coronary flow.
*P < .05 compared with the standard cardioplegia group (ANOVA).

which can mimic induction of natural hibernation when administered to mammalian species. The present study examined the interaction of delta opioid agonists and antagonists and their effect on myocardial functional recovery after global ischemia in a nonhibernating mammalian model.

Methods

Rabbits (2.2-2.7 kg) were anesthetized with sodium pentobarbital (45 mg/kg administered intravenously) and heparinized (700 U/kg administered intravenously). The heart was rapidly excised and immersed in ice-cold physiologic salt solution (PSS), pH 7.4, containing 118.0 mmol/L NaCl, 4.0 mmol/L KCl, 22.3 mmol/L $NaHCO_3$, 11.1 mmol/L glucose, 0.66 mmol/L KH_2PO_4, 1.23 mmol/L $MgCl_2$, and 2.38 mmol/L $CaCl_2$. The aorta was cannulated in the Langendorff mode, and the heart was perfused with PSS equilibrated with 95% O_2-5% CO_2 at 37°C and passed twice through filters with a 3.0-μm pore size. Perfusion pressure was maintained at 80 mm Hg. An incision was made in the left atrium, and a fluid-filled latex balloon was passed through the mitral orifice and placed in the left ventricle. In this established model, the balloon was connected to a pressure transducer for continuous measurement of left ventricular pressure and the first derivative of left ventricular pressure (dP/dt). The venae cavae and the azygos vein were ligated. The pulmonary artery was cannulated to enable timed collection measurements of coronary flow and was connected to an oxygen meter (Diamond, Inc, Ann Arbor, Mich) for continuous measurement of the oxygen partial pressure. The analog signals were continuously recorded on a pressurized ink chart and digitized to an online computer. Cardiac function was characterized by dP/dt and left ventricular developed pressure (LVDP), where LVDP is the isovolumetric pressure generated by the left ventricle defined as peak systolic pressure minus end-diastolic pressure. Myocardial oxygen consumption (MVO_2) was calculated as follows:

$$MVO_2 = CF \times [(Pao_2 - Pvo_2) \times (c/760)],$$

where CF is coronary flow (in milliliters per minute per gram), $Pao_2 - Pvo_2$ is the difference in the Po_2 (in millimeters of mercury) between perfusate and coronary effluent flow, and c is the Bunsen solubility coefficient of O_2 in perfusate at 37°C (22.7 μL O_2 · atm^{-1} · mL^{-1} perfusate). The Po_2 of the perfusate was 665 mm Hg. Coronary flow was measured by performing timed collections of the pulmonary effluent flow with a graduated cylinder. Oxygen extraction was calculated as follows:

$$O_2 \text{ Extraction} = MVO_2/\text{Oxygen content in the perfusate.}$$

Wet and dry myocardial weights and calculation of water content comparatively assessed myocardial edema in each specimen. Wet weight of the heart was determined at the conclusion of each experiment after trimming the great vessels and fat and blot drying with 9-layer cotton gauze. The left ventricular wall was weighed, desiccated for 48 hours at 65°C, and reweighed. Water content was determined by the following formula:

$$(1 - \text{Dry weight/Wet weight})/100\%.$$

A section of the LV was prepared for histopathologic examination. Each of these methods has been previously reported.[1,2]

After Langendorff instrumentation was completed and calibrations had been performed, left ventricular balloon volumes were varied over a range of values to construct modified isovolumic left ventricular function curves. The identical volumes were maintained during baseline and reperfusion conditions. Baseline data were obtained after an equilibration period of 30 minutes. During the baseline period, data were obtained with hearts maintained at 37°C with a water-jacketed organ bath. A Khuri Regional Tissue pH Monitor-intramural pH electrode (Vascular Technology, Chelmsford, Mass) was placed in the left ventricular free wall during infusions to observe pH changes. To observe metabolic changes in tissue nucleotides (adenosine triphosphate, adenosine monophosphate, adenosine diphosphate, and insosine 5′-monophosphate) and nucleosides (adenosine, inosine, hypoxanthine, and xanthine), heart biopsy specimens were rapidly frozen in liquid nitrogen at baseline or at 15 minutes of reperfusion and then lyophilized. Tissue was processed as previously described by our laboratory.[3] High-performance liquid chromatography was performed with a Waters μBondapak C18 column (Milford, Mass). The spectrophotometric detector was set at 254 nm for determination of nucleotides and nucleosides and at 210 nm for measurement of phos-

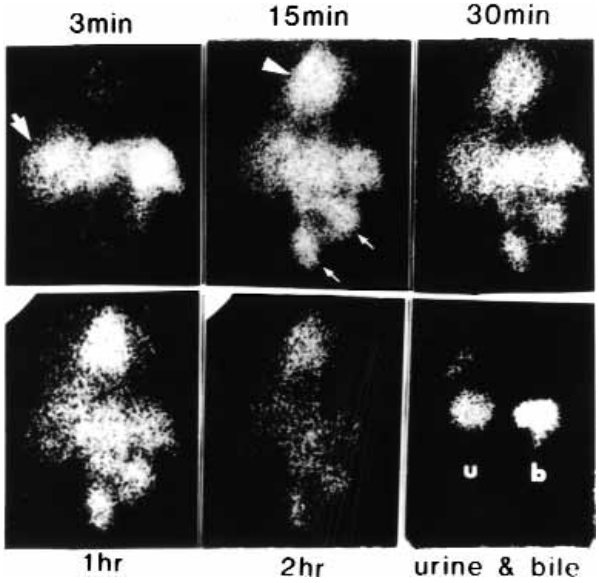

Figure 1. ^{125}I-labeled DADLE timed distribution at 3 minutes, 15 minutes, 30 minutes, 1 hour, and 2 hours after infusion, as well as excretion in the bile *(b)* and urine *(u)* at 2 hours. At 3 minutes, there is initial uptake in the liver *(arrow)*, with subsequent later excretion in the intestine *(small arrows)*. Note the intense early uptake in the myocardium at 15 minutes *(large triangle)*, which persists for 2 hours.

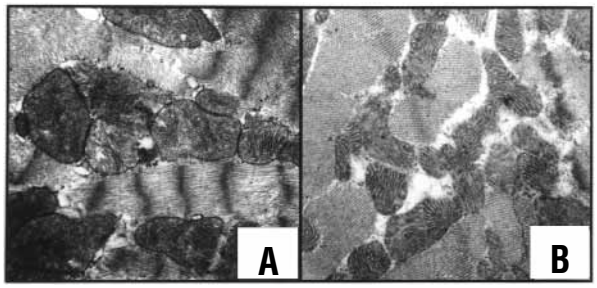

Figure 2. Electron microscopy of cardiac muscle after 2 hours of global ischemia and reperfusion. A, DADLE-treated hearts have unremarkable morphology, with near-normal myofibrillar architecture. (Original magnification 10,000×.) B, Control hearts, even at lower power, reveal numerous ultrastructural changes indicative of ischemia-reperfusion injury, as demonstrated by the loss of myofibrils. The mitochondria are swollen with large amorphous densities, which is suggestive of irreversible injury.

phocreatine. Analysis was performed with Waters Maxima 820 software and NEC Power Mate 1.

The oxygenated PSS infusion was stopped to induce ischemia, and 60 mL of 4°C solution was injected into the aorta at a rate of 1 mL/s to begin the 34°C 2-hour ischemic period. Hearts were randomly assigned to receive either standard cardioplegic solution as controls or preperfusion with a delta opioid agonist, antagonist, or both. Type 1 and type 2 delta receptor agonists and antagonists were investigated individually and in combination before standard cardioplegia-induced ischemia to better elucidate the action of the myocellular delta opioid receptor.

In the individual opioid agonist-antagonist studies, 1 of 2 delta opioid agonists, either the delta-2 D-Ala2-Leu-5-enkephalin (DADLE) or the delta-1 D-pen2,5-enkephalin (DPDPE), were administered at 2 mmol/L over 15 minutes (22.7 mg/20 mL PSS). Other hearts received naltrexone, a nonselective opioid antagonist, alone (as negative controls) in doses of 2×10^{-4} to 2×10^{-6} mmol/L over 15 minutes.

In the combined opioid agonist-antagonist studies, naltrexone was given in vivo 30 minutes before DADLE infusion and coadministered with DADLE infusion for 15 minutes before the 2 hours of ischemia. The involvement of specific delta opioid receptors was further delineated by comparing naltrexone, which blocks both delta 1 and 2; 7-benzylidene-7-dehydronaltrexone hydrochloride (BNTX), a selective delta 1 blocker; or naltriben-methane sulfonate (NTB), a delta 2 blocker, just before DADLE infusions. The doses of opioids and antagonists were selected by means of standard opioid dosing and our prior studies.[1,2,4,5]

The cardioplegic solution contained 109.0 mmol/L NaCl, 25.0 mmol/L KCl, 21.9 mmol/L $NaHCO_3$, 16.0 mmol/L $MgCl_2$, and 0.8 mmol/L $CaCl_2$. On completion of the 2-hour ischemic period, hearts were reperfused with oxygenated PSS at 37°C. To assess the degree of functional recovery in each group, hemodynamic data were recorded every 15 minutes for 45 minutes and compared with baseline data.

In an attempt to identify delta opioid receptor expression in the myocardium, immunohistopathologic staining of anti-delta fluorescent-labeled antibodies was used on myocardial and brain tissue from these rabbits. Additionally, because the specific delta 2 receptor has not been specifically fully characterized and no antibodies to it exist, iodine 125 (^{125}I)–radiolabeled DADLE was infused into animals to identify DADLE/delta 2–receptor distribution in the animal. Blinded evaluation of electron micrographs was used to compare ultrastructural changes in control and opioid-pretreated myocytes. Data are expressed as means ± standard deviation. Statistical analysis for continuous variables was evaluated with analysis of variance (ANOVA; the Scheffé test). All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research.

Results

There were no differences in any metabolic or functional indices during preischemia baseline between groups. Table 1 summarizes the postischemic metabolic and functional recovery results. Compared with standard cardioplegia control hearts, hearts pretreated with DADLE had markedly improved isovolumic left ventricular pressure generation (LVDP: 36 ± 3 vs 65 ± 5 mm Hg, respectively; $P < .01$). Conversely, compared with control hearts, those hearts pretreated with the opioid antagonist naltrexone revealed a functional depression of LVDP (36 ± 3 vs 17 ± 5 mm Hg, $P < .05$). Fittingly, when DADLE and naltrexone were coadministered, the above individual effects on LVDP were negated, and the functional recovery was similar to that of standard cardioplegia (36 ± 3 vs 42 ± 4 mm Hg, P = not significant). The end-diastolic pressures at baseline versus that

at the postischemic recovery period in control, DADLE-treated, and naltrexone-treated hearts were similar (10 vs 14, 13, and 17 mm Hg, respectively; P = not significant).

Figure 1 suggests the presence of specific myocardial DADLE/delta 2 receptors. ^{125}I-radiolabeled DADLE infused intravenously revealed prompt uptake in the heart that persisted for up to 2 hours. Immunohistopathologic staining of anti-delta (nonspecific type 1 and 2) fluorescent-labeled antibodies used on rabbit myocardial and brain tissue revealed scant staining in the heart but good control delta staining in the brain.

Ultrastructural preservation was significantly improved with DADLE pretreatment compared with that seen in control hearts (Figure 2). Blinded assessment denoted better preservation of subcellular structures with less interstitial edema formation and more normal myofibrillar structure in the DADLE group. Opioid untreated control hearts reveal numerous ultrastructural changes indicative of ischemic injury, as demonstrated by myofibrillar loss. Furthermore, the mitochondria were swollen with large amorphous densities, which is suggestive of irreversible injury. There were no significant differences in nucleotide levels or interstitial pH levels between groups because these were both significantly but equally decreased from the preischemic period with or without opioid protection. Furthermore, after Langendorff solution delivery, ischemia, and reperfusion, heart weight and water content were not different between opioid-pretreated and control groups.

Discussion

Mammalian hibernation in endotherms is a unique physiologic state characterized by depressed body temperature, respiration, cardiovascular function, and general metabolism.[1-6] Interestingly, hibernators avoid freezing, remain metabolically regulated, and arouse spontaneously at euthermia with normal levels of systemic function and metabolic activity. Dawe and Spurrier[7] identified the presence of a hibernation trigger-like substance circulating in the plasma of hibernating ground squirrels. When this was administered to nonhibernating animals, a metabolic state similar to hibernation was induced.[8]

Further evidence indicates that this molecule initiates its potent metabolic inhibitory effects through specific membrane opioid receptors and is a small, thermolabile, protease-sensitive, nuclease-insensitive protein.[9,10] These alterations could be blocked or restricted by the infusion of opiate antagonists, such as naloxone and naltrexone.[11,12] Hibernation may specifically involve the delta opioid receptor because mu opioid agonists, such as morphine and morphiceptin, can actually block hibernation.[13,14] These mu agonists will preferentially occupy delta opioid receptor sites.[15] The cellular physiology of mammalian hibernation very closely parallels that of the altered myocyte physiology seen with hypothermic global ischemia. Unlike the poor functional response to prolonged ischemia in cardiac surgery, the drastic subcellular and molecular changes of reduced coronary flow, hypoxia, high-energy phosphate depletion, and volume shifts are well tolerated during hypothermic mammalian hibernation. This physiologic paradox has been the impetus to further elucidate the role of opioids in myocyte metabolism and preservation.

Delta opioid receptors are known to exist in minimal amounts in many peripheral organs, including the heart. Our results in the present study confirm previous reports that general opioid peptides and opioid receptors are found within the myocardium, where they may exert local physiologic effects.[1-6,16-20] A previous report using tritiated DPDPE has demonstrated the existence of delta opioid receptors in the heart.[21] Although only one delta opioid receptor has been cloned, there are believed to be at least two delta subtypes. To identify delta opioid receptor subtypes in the myocardium, we used immunohistopathologic staining of anti-delta 1 fluorescent-labeled antibodies on myocardial and brain tissue from rabbits. This study demonstrated abundant delta 1 opioid receptors in brain tissue but low levels of this particular stain in the heart. Because the delta 2 receptor has not presently been characterized and no antibodies to it exist, we infused ^{125}I-radiolabeled DADLE in an attempt to identify myocardial delta 2 receptors. This study strongly showed ^{125}I-radiolabeled DADLE uptake in the myocardium (Figure 1). This suggests the presence of opioid receptors of the delta 2 subtype in the heart. Moreover, the present study provides further lines of evidence suggesting not only that the delta 2 subtype of opioid receptors exists in the heart but also that their stimulation may mitigate the functional response to myocardial ischemia. First, the selective delta 1 ligand DPDPE was relatively inactive, and the anti-ischemic action of DADLE, which acts at both delta subtypes of opioid receptors, could only be antagonized by the selective delta 2 antagonist NTB and not the delta 1 antagonist BNTX.[22] Finally, the existence of separate subtypes of delta opioid receptors has been further postulated from analgesic testing in animals, in which the analgesic effect of only delta 2–selective agonists were blocked by antisense oligodeoxynucleotides directed against cloned delta opioid receptors.[23]

The current study revealed that the use of the delta opioid DADLE profoundly improved myocardial functional recovery after ischemia. When administered alone, naltrexone (an opioid antagonist) did not improve and even decreased postischemic myocardial function. This may relate to an inhibition of a baseline level of opioid receptor activation. Additionally, when administered with DADLE, naltrexone blocked its cardioprotective effects in a dose-response manner. These results suggest that naltrexone, with a higher affinity than DADLE, is acting as a competitive delta receptor antagonist and thus diminishing the protective effects of exogenously administered DADLE.

The involvement of the specific delta opioid receptors in myocardial preservation was further delineated by comparing

the universal opioid antagonist naltrexone and the highly selective delta 1 blocker BNTX or the delta 2 blocker NTB just before DADLE infusions. Our results, which demonstrated blocking of myocardial tolerance to ischemia with a specific delta 2 antagonist but not a delta 1 antagonist, provide indirect evidence that delta 2 opioid receptors are specifically involved in myocardial protection.

These results demonstrated improved myocardial tolerance to ischemia in a pharmacologic receptor agonist-antagonist manner, thereby providing direct evidence that delta opioid receptors are involved in myocardial protection. This finding builds on the suggestion by our laboratory's prior results that opioids confer myocardial protection.[1-6] Other investigators have put forth further corroborating evidence by using the delta opioid DADLE in multiorgan autoperfusion systems to extend organ survival time.[24,25] Furthermore, successful lung transplants from DADLE-treated organ blocs after 24 hours of ex vivo preservation have been performed.[26] Finally, recent reports have provided indirect evidence that delta opioid receptors are involved in the ischemic preconditioning in the rat and dog myocardium.[27,28]

Delta opioid receptors belong to the transmembrane guanine nucleotide binding protein (G protein)–linked superfamily of receptors. This binding is known to alter intracellular signal transduction pathways, which is thought to liberate the cardioprotective effects of potassium-adenosine triphosphate–linked potassium channels. However, the precise mechanism behind the anti-ischemic action of delta opioids remains elusive. DADLE has been shown to increase the level of inositol from 1,4,5-triphosphate release of Ca^{2+} from sarcoplasmic reticulum in the rat ventricular cardiac myocytes.[29] Opioids may also alter contractile protein regulation in response to the local metabolic environment. Although opioid receptors are linked to adenylate cyclase activation, it is important to note that delta opioids do not exert their action through an inotropic mechanism. Data from our laboratory revealed that when DADLE was administered to normal beating nonischemic rabbit hearts in concentrations from 0.01 to 100 mmol/L, there was no inotropic effect and no alteration in pH or MVO_2 levels.

In conclusion, it is tempting to propose that delta opioid receptors may represent one of nature's protective mechanisms for tissue survival. Therefore, further elucidation of their cardioprotective mechanisms in the ischemic and failing myocardium is warranted.

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