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Role of δ-opioid receptor agonists on infarct size reduction in swine

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Sigg, Daniel C., James A. Coles, Jr., Peter R. Oeltgen, and Paul A. Iaizzo. Role of δ-opioid receptor agonists on infarct size reduction in swine. *Am J Physiol Heart Circ Physiol* 282: H1953–H1960, 2002; 10.1152/ajpheart.01045.2001.—Opioids are involved in cardiac ischemic preconditioning. Important species differences in cellular signaling mechanisms, antiarrhythmic, and antistunning effects have been described. The role of the δ-opioid receptor activation in swine remains unknown. Forty minutes before a 45-min occlusion and 180-min reperfusion of the left anterior descending coronary artery, open-chest, pentobarbital-anesthetized swine received either *1*) saline (controls); *2*) [D-Ala^2,D-Leu^5]enkephalin (DADLE); *3*) [D-Pen^{2,5}]enkephalin (DPDPE); *4*) deltorphin-D, a novel δ_2-opioid agonist; or *5*) ischemic preconditioning (IP). Assessed were *1*) infarct size to area at risk (IS, triphenyltetrazolium staining), *2*) regional and global myocardial function (sonomicrometry, ventricular pressure catheters), and *3*) arrhythmias (electrocardiogram analyses). It was found that DPDPE and deltorphin-D pretreatment reduced IS from 64.7 ± 5 to 36.5 ± 6% and 27.4 ± 11% ($P < 0.01$), respectively, whereas DADLE had no effect (66.8 ± 3%). Both IP and DADLE had a proarrhythmic effect ($P < 0.01$). However, no differences in global or regional myocardial function or arrhythmia scores were observed between groups. This suggests that δ-receptor-specific opioids provide cardioprotection in swine.

cardioprotection; myocardial ischemia; regional myocardial function; arrhythmia; κ-opioid receptor

A GREAT DEAL OF INTEREST has focused on the ischemic preconditioning (IP) phenomenon, and many endogenous mediators such as adenosine, bradykinin, and opioids have been identified as beneficial mediators for acute ischemic preconditioning. Classical IP procedures have been shown to induce myocardial stunning (18) and may be associated with other ischemia-related complications such as arrhythmias (16). Therefore, intuitively, pharmacological preconditioning seems potentially more advantageous. In particular, the involvement of δ-opioid receptors and receptor agonists (δ-opioids) appears promising for several reasons: *1*) opioids have been shown to be involved in IP in various species (22, 28, 32, 33), including humans (3, 37); *2*) δ-opioid receptor activation is one of the possible pathways implicated in mammalian hibernation (20, 25); *3*) IP by δ-opioids is not limited to the heart (9, 21, 24, 41, unpublished observations on ischemic protection of the brain by Dr. Oeltgen, skeletal muscle by our laboratory); *4*) δ-opioid receptors are expressed on human myocardium (3); and *5*) these agents may induce potent analgesic effects (2, 17). However, in humans it is not known whether opioids specifically reduce infarct size. Yet, a role of endogenous opioid receptor activation in preconditioning in humans has been suggested, as naloxone was shown to block the beneficial effects of IP on S-T segment changes during percutaneous transluminal coronary angioplasty (PTCA) (37), and as preconditioning with the δ-opioid agonist [D-Ala^2,D-Leu^5]-enkephalin (DADLE) improved postischemic function of isolated atrial trabeculae (3).

Important species differences have been described for not only intracellular signaling mechanisms (38), but also for both the opioid receptor subtypes involved (6, 7, 14, 29, 31, 40) and for the opioid dosages needed to elicit cardioprotection (22, 28). More specifically, in rats, endogenous and exogenous activation of the δ_1-opioid receptor subtype reduced infarct size in ischemic and opioid preconditioning via G proteins and potassium-dependent ATP channels (14, 15, 28–32), whereas conflicting effects of κ-receptor activation have been reported in this species alone (14, 29, 40). Furthermore, pharmacological preconditioning with δ-opioid agonist DADLE and δ_1-agonist TAN-67 has been shown to specifically reduce infarct size in rats (14, 15, 31), whereas, to our knowledge, no such information exists in swine or other species. Nevertheless, a general role of endogenous opioids in IP has been suggested in rabbits and in swine, because naloxone reportedly abolished infarct size reduction following IP (22, 33). Globally ischemic isolated rabbit and porcine hearts preconditioned with DADLE have been reported to elicit improved myocardial postischemic function, but actual infarct sizes were not measured (4, 6, 7, 36). Therefore, it remains unclear whether reduced necrosis, attenuation of stunning, or both were critical for

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the observed functional benefits. We recently showed that preconditioning with DADLE or morphine improved postischemic function after global hypothermic ischemia without attenuating cardiac enzyme leakage in isolated working swine hearts (36). One can speculate that DADLE, morphine, and other nonspecific opioid agonists may activate myocardial κ-opioid receptors, thereby inducing an antipreconditioned state as suggested by Aitchison et al. (1). Importantly, the effects of opioid preconditioning on regional postischemic dysfunction and load-independent parameters of global ventricular function have not been investigated in any species.

Of additional interest, in rats, antiarrhythmic effects (reduced ischemia-related arrhythmias) of classical IP (35) and of preconditioning with δ_1-opioid agonist TAN-67 have been demonstrated (14). Conversely, in swine, IP has been shown to be arrhythmogenic (16), and, accordingly, the opioid antagonist naloxone has been reported to decrease ischemia-related arrhythmias (5). This suggests a proarrhythmic activity of opioids in this species. However, it is unknown whether preconditioning with specific δ-opioids reduces the occurrence of sublethal arrhythmias in swine.

The specific aims of the present study were to evaluate the cardioprotective effects of exogenously administered ∂-opioids: [D-Pen^{2,5}]enkephalin (DPDPE), a δ_1-specific opioid agonist; deltorphin-D, a novel possibly δ_2-specific agonist; and DADLE, a primary δ_1- and δ_2-agonist. Specifically, we compared the effects of opioid preconditioning with those of classical IP and to controls. To do so, as the main outcome parameters in an acute coronary occlusion model of swine, we determined infarct size, regional and global myocardial functions, and the incidences of lethal and sublethal arrhythmias.

METHODS

This study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* [Department of Health and Human Services Publication No. (NIH) 85-23, Revised 1985] after approval from the Institutional Animal Care and Use Committee of the University of Minnesota.

Surgical preparation. Yorkshire, non-Pietrian swine (37 ± 1 kg, means ± SE) were sedated with midazolam intramuscularly (2 mg/kg) and anesthetized with intravenous pentobarbital sodium (20 mg/kg) followed by a continuous infusion (5–20 mg·kg^{-1}·h^{-1}). After endotracheal intubation, ventilation (2:1 air to oxygen mixture) was adjusted to maintain an arterial P_{CO_2} of 40 ± 2 mmHg, and core temperature was maintained at 38 ± 0.5°C using convective air warming as needed (Bair Hugger, Augustine Medical; Eden Prairie, MN). Two Mikro-Tip catheter transducers (5-Fr, model MPC-500, Millar Instruments; Houston, TX) were placed via the right carotid artery into the ascending aorta and the left ventricle. Two femoral artery cannulas (*A. femoralis superficialis*) were inserted for blood pressure monitoring and blood sampling (blood gas analysis, myocardial blood flow). A medial sternotomy was performed, exposing the heart and the major vessels. A four-suture pericardial cradle was used to suspend the heart, and a myocardial thermocouple probe was inserted between the epicardium and pericardium. The left atrial appendage was cannulated for microsphere and patent blue dye injections. The aortic and left anterior descending (LAD) coronary artery flows were measured via transonic flow probes (Transonic Systems; Ithaca, NY) placed on the ascending aorta and on the LAD distal to the planned occlusion site. Two-millimeter ultrasound crystals (Sonometrics; London, Ontario, Canada), placed on the end points of the two major axes of the left ventricles, were used to determine left ventricular volumes and pressure-volume relationships (maximal elastance, E_{max}, and preload recruitable stroke work, PRSW) during temporary occlusion of the inferior vena cava. Additionally, regional left ventricular function was estimated by measuring segment shortening. This was accomplished by placing five ultrasound crystals in a linear manner along the anterior surface of the left ventricle forming four adjacent segments in the short axis, ~1 cm apart. They were positioned in an array so that the first segment was always located in the center of the area at risk and the most lateral segment was consistently in the area at nonrisk. All data were acquired with the Sonosoft software (Sonometrics; London, Ontario, Canada), and postacquisition analysis was performed using Cardiosoft software (Sonometrics).

In each heart, a 2- to 3-mm segment of the LAD coronary artery was dissected distal to the first diagonal branch for occlusion and placement of the coronary flow probe (see above). The animals were fully heparinized following surgical preparation and throughout the subsequent experimental protocol (300 IU/kg intravenous bolus of heparin followed by an infusion of 67 IU·kg^{-1}·h^{-1}).

Measurement of infarct size and risk area. On completion of the reperfusion period, the LAD was reoccluded, and patent blue dye was injected via the left atrium to differentiate the ischemic area (area at risk) from the nonischemic area (area at nonrisk). After being frozen at −20°C overnight, hearts were sliced into 4-mm transverse slices. The slices were then incubated with 1% triphenyltetrazolium chloride in phosphate buffer (pH 7.4) at 37°C for a period of 10 min. Triphenyltetrazolium chloride forms a red formazan derivative when reacting with viable tissue, whereas necrotic tissue is pale white once fixed in 10% formalin. Area at risk, area at nonrisk, and infarct size were assessed in a blinded fashion using computer-assisted planimetry (UTHSCSA ImageTool software, University of Texas Health Science Center; San Antonio, TX).

Regional myocardial blood flow. Regional myocardial blood flow (RMBF) to the area at risk and area at nonrisk were assessed to determine collateral blood flow during ischemia. Colored microspheres (E-Z TRAC 15 μm diameter blue Ultraspheres, Interactive Medical Technologies; Irvine, CA) were injected into the left atrium while a reference blood sample was simultaneously drawn to determine reference blood flow during 30 min of ischemia. Subsequently, the number of microspheres was assessed microscopically from the reference blood sample, the area at risk, and the area at nonrisk. Reference blood flow was calculated as the difference between syringe weights pre- and postwithdrawal, corrected for blood density (1.05 g/ml), divided by collection time. Routine tissue and blood processing were completed (according to the procedural instructions by Interactive Medical Technologies). Blood flow was calculated using the formula: $RMBF = Q_b \times C_t/C_b$, where Q_b is reference blood flow, C_t is number of microspheres in tissue normalized per gram wet weight, and C_b is number of microspheres of the blood reference sample (40).

Arrhythmia assessment. A standard peripheral lead electrocardiogram was used to monitor arrhythmias upon reperfusion, and analysis was completed using the Ponemah Physiology Platform Version 3.1 software (Gould Instrument

Systems; Valley View, OH). The following modified scoring system was used to quantify arrhythmias by a person blinded to the experimental protocol: 0, <10 premature ventricular contractions (PVC) in 9 min; 1, 10–50 PVC in 9 min; 2, >50 PVC in 9 min; 3, 1 episode of ventricular fibrillation (VF) in 9 min; 4, 2–5 episodes of VF in 9 min; and 5, >5 episodes of VF in 9 min, modified from Curtis et al. (12) and Fryer et al. (14).

VF was treated by defibrillation shocks of 50 J administered via internal paddles and repeated if necessary. If the animal did not recover a spontaneous atrioventricular rhythm after 1 min of continuous VF, it was considered intractable and excluded from the study.

Experimental protocol. The experimental protocol is illustrated in Fig. 1. After the surgery was completed, animals were allowed to stabilize for ≥20 min. The animals were randomly assigned into the following six groups, which differed only in their preconditioning protocol (preconditioning phase P0-P40). The control group (n = 7) received intravenous 0.9% saline injection (10 ml) during preconditioning phase. The DPDPE group (n = 6) received intravenous injection of 1 mg/kg (in 10 ml) [D-Pen^{2,5}]enkephalin, a specific δ_1-opioid receptor agonist, over two times 10 min (40 and 20 min before coronary occlusion, respectively). The deltorphin-D group (n = 4) received intravenous injection of 1 mg/kg deltorphin-D, a novel putative δ_2-opioid receptor agonist using the same infusion protocol as the DPDPE group. The DADLE group (n = 7) received 1 mg/kg [D-Ala^2,D-Leu^5]enkephalin, a δ_1- and δ_2-specific opioid agonist using the aforementioned infusion protocol. The ischemic preconditioning group (n = 3) consisted of two 10-min cycles of coronary occlusion followed by 10 min of reperfusion 40 and 20 min before LAD occlusion. Finally, in the DADLE + nor-binaltorphimine group (nor-BNI; n = 3), nor-BNI, a selective κ-antagonist, was administered 2 h before DADLE intravenously at a dose of 1.5 mg/kg. DADLE was administered as described in the DADLE group.

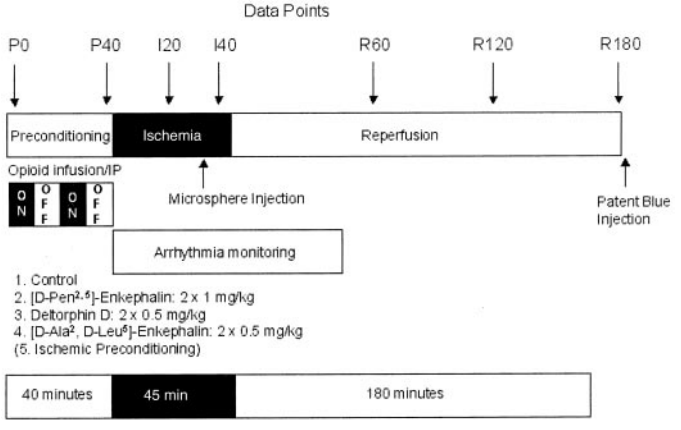

Fig. 1. Shown is the timeline of the experimental protocol. Study was divided into a preconditioning phase, an ischemia phase, and a reperfusion phase. Hemodynamic data were collected at the time points indicated (see text for details). Importantly, drug infusions were administered during the preconditioning phase over 10 min, followed by a drug-free (or reperfusion in ischemic preconditioning) interval of 10 min, and then repeated once. Subsequent assessment of arrhythmias was performed using the continuous electrocardiogram data obtained during coronary occlusion (ischemia) and during the first 45 min of reperfusion (90 min total). Microspheres were injected at 30 min of ischemia to assess regional myocardial blood flow (collateral blood flow) in the area at risk. Finally, area at risk was assessed at the end of the protocol.

Subsequently, the LAD was occluded for 45 min (ischemia phase, I0–I45) with an arterial occluder (Sklar Vascular Size 2 Single Clamp, Sklar Instruments; West Chester, PA). The LAD clamp was then removed, and the ischemic myocardium was reperfused for 180 min (reperfusion phase, R0–R180). Hemodynamic data, electrocardiogram analysis, and RMBF were assessed at the indicated time points (Fig. 1).

Data analysis and statistics. Data are reported as means ± SE. Data from all groups were analyzed using repeated-measures ANOVA and Fisher's protected least-significant-difference (PLSD) test as a post hoc test if significant differences were detected between groups. Nonrepetitive measurements such as infarct size were analyzed using one-way ANOVA and Fisher's PLSD test. For testing significance of excluded animals, Fisher's exact test was employed.

RESULTS

Thirty swine were enrolled into the study. Six animals (20%) were excluded during the study protocol due to either *1*) intractable VF (one control, three IP, and one DADLE animal); *2*) an extensive area at risk (1 DPDPE); or *3*) excessive bleeding (1 control).

After losing three animals undergoing acute IP due to intractable VF during the preconditioning phase, no further animals were enrolled into the IP group. The included study animals (n = 25) consisted of 6 controls, 6 DPDPE, 4 deltorphin-D, 6 DADLE, and 3 DADLE + nor-BNI.

No significant differences between animal weight, arterial P_{CO_2}, pH, core or myocardial temperatures (average myocardial temperature 37.9 ± 0.07°C), total pentobarbital dosage, or total fluid administration (both normalized per kg) were detected between any of the experimental groups. These parameters were determined for those animals that completed the entire protocol.

Infarct size. Animals pretreated with either DPDPE or with deltorphin-D had a significantly lower infarct size compared with controls and DADLE-pretreated animals ($P < 0.01$, Fig. 2*A*). In a subgroup, coadministration of κ-opioid receptor nor-BNI and DADLE reduced infarct size significantly compared with DADLE alone or controls ($P < 0.05$, Fig. 2*A*). The area at risk of the left ventricle averaged 22.6 ± 0.9% (n = 25) and was not different between groups (Fig. 2*B*).

Hemodynamic findings. Hemodynamic findings are summarized in Table 1 and Fig. 3. Baseline values did not significantly differ between groups.

During ischemia and on subsequent reperfusion, significant decreases in global and regional left ventricular function were observed (Table 1, Fig. 3). There were no significant differences in global hemodynamic performance between groups during ischemia and reperfusion. However, it was noted that PRSW and positive first derivative of pressure (dP/dt) decreased significantly versus baseline in both DPDPE and deltorphin-D groups (repeated-measures ANOVA), but not in controls, yet direct group comparison was not significant (see Table 1).

Regional myocardial blood flow. The average blood flow to area at nonrisk at 30 min ischemia was 1.5 ±

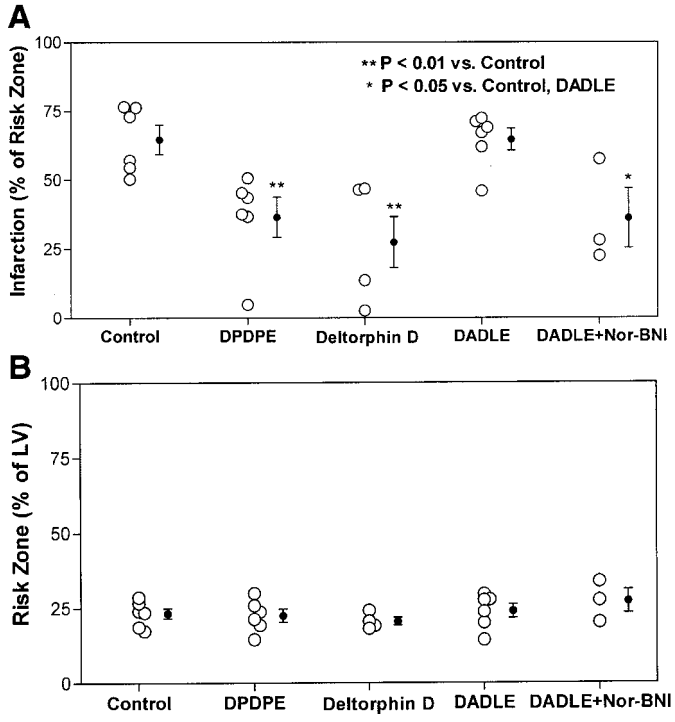

Fig. 2. *A*: infarct sizes (% area at risk) of controls and animals preconditioned with [D-Ala^2,D-Leu^5]enkephalin (DADLE), [D-Pen^{2,5}]enkephalin (DPDPE), deltorphin-D, or DADLE and nor-binaltorphimine (DADLE+ nor-BNI). Infarct sizes were significantly reduced in the DPDPE group ($P < 0.01$ vs. control and vs. DADLE), the deltorphin-D group ($P < 0.01$ vs. control and DADLE), and the DADLE+ nor-BNI group ($P < 0.05$ vs. control and DADLE). *B*: areas at risk [% of left ventricle (LV)] of controls and animals preconditioned with DADLE, DPDPE, deltorphin-D or DADLE+nor-BNI. No differences were detected between groups. Open circles, actual individual experiments; closed circles, means; vertical bars, ±SE.

0.2 ml·min^{-1}·g^{-1} ($n = 24$), and there were no differences between groups. Additionally, no significant collateral blood flow was detected in any of the animals (transmural blood flow area at risk <0.05 ml·min^{-1}·g^{-1}; $n = 24$).

Arrhythmia analysis. One control (1 of 7), three ischemic preconditioned (3 of 3), and one DADLE preconditioned animal (1 of 7) had to be excluded due to intractable VF, whereas this did not occur in any of the DPDPE- or deltorphin-D-treated animals (0 of 10). It was also noted that the incidence of intractable VF was significantly higher in ischemically preconditioned animals compared with DPDPE- and deltorphin-D-treated animals ($P < 0.01$) and was marginally higher than observed in controls ($P = 0.08$), implying a proarrhythmic effect of IP.

Nevertheless, of the included animals, the arrhythmia scores during 45 min of ischemia and the first 45 min of reperfusion were not different between groups with the exception of increased arrhythmia incidence in the DADLE group during ischemia compared with controls ($P < 0.05$, Fig. 3), whereas nor-BNI pretreatment abolished the proarrhythmic effect of DADLE ($P < 0.05$ vs. DADLE; Fig. 3). Similarly, the number of total PVCs was higher in DADLE-pretreated animals during ischemia ($P < 0.01$, data not shown), whereas no differences were detected during the reperfusion period. The incidence of VF did not differ significantly between groups (excluding the IP group).

Discussion

This study provides the first evidence that the specific δ-receptor opioid agonists DPDPE and deltorphin-D both decrease infarct size in swine hearts at clinically relevant doses. However, no differences in sublethal arrhythmias were detected between either the DPDPE- or deltorphin-D-preconditioned animals relative to controls. Additionally, postischemic regional and global left ventricular function overall was not significantly improved with opioid preconditioning compared with controls. DADLE did not confer cardioprotection in this model and was associated with increased arrhythmogenesis during ischemia. Interestingly, the coadministration of a κ-antagonist and DADLE was cardioprotective and the arrhythmogenic effect of DADLE alone was completely abolished. Nevertheless, the methods employed here were considered highly reproducible: similar areas of risk and functional parameters were observed. Furthermore, important variables such as myocardial temperatures, fluid, and anesthetic administration were carefully monitored and controlled. Finally, the protocols used for agent administration were considered to be of clinical relevance.

Open-chest, anesthetized coronary occlusion model. The clinical relevance and potential limitations of open-chest, anesthetized swine models have been described elsewhere (39). The swine model was chosen because it very closely resembles the human physiology and anatomy, i.e., lack of coronary collateral flow, similar coronary and heart anatomy, and similar timing of infarct development (39). In the present study, midazolam sedation and pentobarbital anesthesia were employed because these drugs have not been reported to be myocardial protective [such as volatile anesthetics (11) or opioids (4, 28, 30)] and they were not considered to block myocardial protection [such as ketamine (23)].

It should be noted that DPDPE was administered at an intravenous dose of 2 mg/kg; for comparison, intravenous doses of up to 56 mg/kg were previously given to mice, which increased hypoxic tolerance (21). Similarly, the doses of DADLE at 1 mg/kg intravenously have been employed in mice (21), rats (14), and dogs (9). The dosing of the novel peptide deltorphin-D was based on dose-response myocardial and cerebral protection experiments recently performed in rodents (unpublished observations by P. R. Oeltgen).

Infarct size-limiting effects of opioids. Although infarct size-limiting effects of opioid preconditioning on the heart have been described in small mammalians (rats and rabbits) (14, 22, 28, 30, 31), evidence of such effects in large mammalians and humans has been minimal or indirect. For example, preconditioning with

Table 1. *Systemic hemodynamics and blood flow in control, DPDPE, deltorphin-D, and DADLE groups*

			Ischemia		Reperfusion		
	Baseline	Preischemia	20 Min	40 Min	60 Min	120 Min	180 Min
HR, beats/min							
Control	91 ± 9	92 ± 6	91 ± 8	98 ± 10	118 ± 5*	116 ± 4*	122 ± 5*
DPDPE	97 ± 9	93 ± 6	93 ± 6	92 ± 4	130 ± 5*	135 ± 11*	135 ± 12*
Deltorphin-D	77 ± 4	96 ± 1*	98 ± 1*	101 ± 1*	122 ± 5*	117 ± 3*	123 ± 6*
DADLE	90 ± 8	99 ± 2	114 ± 18	126 ± 15*	135 ± 8*	141 ± 10*	142 ± 14*
LVSP, mmHg							
Control	101 ± 5	102 ± 4	94 ± 3	95 ± 3	89 ± 3*	84 ± 4*	84 ± 4*
DPDPE	114 ± 6	112 ± 4	104 ± 4	104 ± 5	85 ± 6*	96 ± 4*	94 ± 3*
Deltorphin-D	116 ± 3	111 ± 4	96 ± 6*	87 ± 5*	80 ± 7*	82 ± 4*	84 ± 3*
DADLE	106 ± 4	110 ± 5	99 ± 10	96 ± 13	78 ± 11*	92 ± 5*	86 ± 4*
LVEDP, mmHg							
Control	8 ± 1	6 ± 1	10 ± 1*	9 ± 1	8 ± 1	7 ± 1	6 ± 1
DPDPE	10 ± 2	8 ± 2	11 ± 2	10 ± 2	8 ± 1	8 ± 2	8 ± 2
Deltorphin-D	10 ± 1	9 ± 2	10 ± 1	8 ± 1	8 ± 1	8 ± 2	11 ± 2
DADLE	10 ± 2	9 ± 2	13 ± 2	10 ± 2	6 ± 2*	8 ± 2	8 ± 2
dP/dt_{max}, mmHg/s							
Control	1,573 ± 107	1,425 ± 104	1,443 ± 107	1,361 ± 85*	1,243 ± 87*	1,185 ± 56*	1,140 ± 45*
DPDPE	1,511 ± 153	1,454 ± 127	1,292 ± 84*	1,247 ± 108*	1,082 ± 76*	1,050 ± 78*	1,060 ± 72*
Deltorphin-D	1,369 ± 88	1,208 ± 92	1,095 ± 96*	941 ± 68*	960 ± 95*	1,109 ± 48*	1,086 ± 39*
DADLE	1,512 ± 114	1,462 ± 122	1,217 ± 140	1,206 ± 56	1,106 ± 199*	1,471 ± 229	1,392 ± 222
Tau, ms							
Control	36 ± 1	35 ± 2	40 ± 3	40 ± 2	42 ± 2	40 ± 1	40 ± 2
DPDPE	43 ± 5	38 ± 3	44 ± 3	42 ± 3	46 ± 4	45 ± 3	43 ± 3
Deltorphin-D	38 ± 4	39 ± 2	44 ± 2	42 ± 2	40 ± 1	38 ± 2	37 ± 2
DADLE	39 ± 3	40 ± 4	47 ± 4	48 ± 6	61 ± 12*	39 ± 2	38 ± 2
CBF, ml/min							
Control	12 ± 1	13 ± 1	0 ± 0*	0 ± 0*	28 ± 2*	18 ± 1*	12 ± 1
DPDPE	13 ± 2	14 ± 2	0 ± 0*	0 ± 0*	32 ± 6*	25 ± 4*	15 ± 3
Deltorphin-D	13 ± 1	16 ± 3	0 ± 0*	0 ± 0*	26 ± 3*	19 ± 2	14 ± 2
DADLE	12 ± 2	14 ± 1	0 ± 0*	0 ± 0*	22 ± 7*	19 ± 5	11 ± 3
Mean ABF, $l \cdot min^{-1} \cdot kg\ body\ wt^{-1}$							
Control	0.08 ± 0.00	0.07 ± 0.00*	0.06 ± 0.00*	0.06 ± 0.00*	0.06 ± 0.00*	0.05 ± 0.01*	0.05 ± 0.00*
DPDPE	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00*	0.06 ± 0.01*	0.05 ± 0.01*
Deltorphin-D	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01*	0.05 ± 0.00*	0.06 ± 0.01*	0.06 ± 0.01
DADLE	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01*	0.06 ± 0.01*	0.05 ± 0.00*	0.06 ± 0.01*	0.05 ± 0.01*
E_{max}							
Control	3.5 ± 0.8	3.6 ± 0.6	2.6 ± 0.3	2.8 ± 0.4	3.7 ± 0.4	5.0 ± 0.8*	4.8 ± 0.5
DPDPE	3.8 ± 0.7	3.3 ± 0.5	2.7 ± 0.3	2.5 ± 0.3	4.0 ± 0.4	4.6 ± 0.9	4.7 ± 0.8
Deltorphin-D	2.5 ± 0.2	3.0 ± 0.3	2.5 ± 0.3	2.7 ± 0.1	2.7 ± 0.4	3.5 ± 0.5*	4.2 ± 0.7*
DADLE	3.9 ± 0.3	3.1 ± 0.4	2.7 ± 0.3	3.1 ± 0.4	5.2 ± 0.7*	6.0 ± 1.1*	5.9 ± 1.2*
PRSW, mmHg							
Control	58 ± 5	62 ± 6	53 ± 5	53 ± 4	51 ± 4	46 ± 6*	40 ± 5*
DPDPE	67 ± 10	66 ± 5	49 ± 4*	49 ± 3*	48 ± 6*	46 ± 8*	42 ± 6*
Deltorphin-D	51 ± 1	47 ± 4	40 ± 2*	38 ± 3*	40 ± 4*	38 ± 3*	36 ± 2*
DADLE	57 ± 1	64 ± 3	50 ± 2	46 ± 2*	43 ± 3*	43 ± 3*	41 ± 4*
SS AAR, %							
Control	20 ± 1	18 ± 1	−5 ± 1*	−4 ± 1*	−4 ± 1*	−4 ± 1*	−4 ± 1*
DPDPE	16 ± 3	18 ± 1	−6 ± 1*	−4 ± 1*	−7 ± 0*	−7 ± 1*	−7 ± 1*
Deltorphin-D	18 ± 1	16 ± 1	−5 ± 2*	−3 ± 1*	−6 ± 1*	−5 ± 1*	−5 ± 1*
DADLE	17 ± 3	14 ± 2	−6 ± 2*	−4 ± 1*	−3 ± 1*	−4 ± 1*	−4 ± 1*
SS NAR, %							
Control	18 ± 3	17 ± 3	18 ± 3	15 ± 3	13 ± 2*	13 ± 1*	12 ± 1*
DPDPE	17 ± 2	15 ± 2	16 ± 1	16 ± 2	14 ± 1	11 ± 2*	10 ± 3
Deltorphin-D	17 ± 1	16 ± 1	18 ± 2	16 ± 2	14 ± 2*	13 ± 1*	13 ± 1
DADLE	10 ± 3	10 ± 3	8 ± 3	9 ± 1	8 ± 3	10 ± 3	8 ± 2

Values are means ± SE. Control group, no preconditioning; DPDPE group, [D-Pen^{2,5}]-enkephalin preconditioning before 45 min of ischemia; Deltorphin-D, deltorphin-D before 45 min of ischemia; DADLE, [D-Ala^2, D-Leu^5]enkephalin before 45 min of ischemia; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt_{max}, maximum of the first derivative of left ventricular pressure; tau, relaxation time constant; CBF, coronary blood flow in the LAD; mean ABF, mean aortic blood flow; E_{max}, maximum elastance of the left ventricle; PRSW, preload recruitable stroke work of the left ventricle; SS AAR, segment shortening in segment within area at risk (%); SS NAR, segment shortening in nonischemic segment (%). *$P < 0.05$ vs. baseline.

DADLE has been reported to mimic IP in isolated human atrial trabeculae (3), and naloxone was reported to both block the beneficial effects of repeated PTCA balloon inflations on S-T segment changes in humans (37) and to block the specific infarct-limiting effects of IP in swine (33).

To our knowledge, this is the first study to demonstrate that preconditioning with DPDPE or deltor-

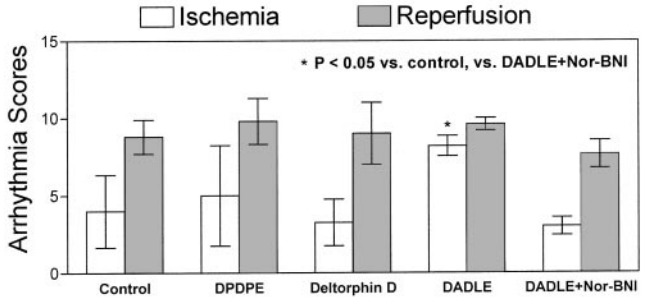

Fig. 3. Mean (±SE) arrhythmia scores during coronary occlusion (ischemia) and the first 45 min of reperfusion in control animals and animals preconditioned with either [D-Pen^{2,5}]enkephalin (DPDPE), deltorphin-D, [D-Ala^2,D-Leu^5]enkephalin (DADLE), or DADLE and nor-binaltorphimine (DADLE+ nor-BNI) are shown. A cumulative score from 2 sets (ischemia and reperfusion) of 5 consecutive 9-min intervals is shown (modified from Refs. 12, 14). In DADLE animals, a significantly increased incidence of ventricular arrhythmias was observed during ischemia ($P < 0.05$). When nor-BNI was administered before DADLE, this proarrhythmic effect was abolished. Otherwise, no significant differences were detected between groups.

phin-D reduces myocardial infarct size in the swine heart, and the first report to indicate a possible role of the δ-opioid receptor in this species. Yet, these findings are in accordance with the observations that δ_1-opioid receptor agonists mediate myocardial protection in rats (29, 31). However, they should be regarded as contradictory to findings in isolated rabbits hearts, where DPDPE did not confer myocardial protection (6, 7).

Another important result of the present study was the lack of protective effects of DADLE. Nevertheless, this finding is in accordance with previous work from our laboratory where DADLE did not reduce cardiac enzyme leakage in an isolated working swine heart model following global ischemia (36). Species differences may be very important relative to the pharmacoprotective effects of DADLE, because this compound has been reported to reduce infarct size at the very same dose in rats (14); DADLE pretreatment reduced infarct size when given at nanomolar doses; however, there were less beneficial effects at higher (micromolar) doses (1). Conversely, in rabbits, high-dose (2 mmol/l) DADLE administration before 2 h of global ischemia improved postischemic myocardial function in isolated hearts; however, infarct size was not measured (7). Cardioprotective effects mediated by DADLE were thought to be induced by specific δ_2-opioid receptor stimulation in rabbits, again eluding to the existing species differences (6). Interestingly, infarct sizes were increased compared with controls when the δ-opioid antagonist naltrindole was given in conjunction with DADLE, suggesting an "antipreconditioning" effect via κ-opioid receptor stimulation (1). Whereas the reason for the lack of cardioprotection by DADLE in the current study is unknown, it may be speculated that in swine, DADLE activates the myocardial κ-opioid receptor at the doses used in the current study, inducing a similar "antipreconditioned" state (1). In support of this, we report here that the coadministration of a κ-antagonist and DADLE conferred significant reductions in infarct sizes. Furthermore, our laboratory is currently investigating the opioid receptor expression and localization and possible colocalization of κ-, δ-, and μ-receptors in porcine myocardium, as well as further cardioprotective roles of different opioid receptor subtypes and their interactions in the currently employed coronary occlusion model.

Myocardial function. Previously, Qiu et al. (27) reported that IP resulted in improved regional myocardial function after 40 min of coronary occlusion in swine. However, many other studies have shown that there is no immediate functional improvement with IP after coronary occlusions lasting more than 30 min, possibly due to myocardial stunning; the topic was critically reviewed in a paper by Cohen and colleagues (10). Intuitively, pharmacological preconditioning may be considered more clinically applicable than IP in terms of attenuation of stunning, because the IP procedure can induce stunning by itself (26). However, following 45 min of coronary occlusion, the present study did not provide evidence that preconditioning with opioids readily attenuates either acute regional dysfunction or improves global function compared with controls. It should be noted that there were trends that suggest that opioid preconditioning depressed systolic performance during ischemia because both DPDPE and deltorphin-D showed a significant decline in maximal dP/dt and PRSW during ischemia (I20, I40), whereas these parameters did not change in controls and DADLE animals. Our laboratory is currently investigating this phenomenon as a possible partial mechanism of opioid preconditioning. Yet, there may be a role of opioids in attenuating stunning and/or improving global myocardial function, for example *1*) by a delayed preconditioning mechanism, *2*) in acute preconditioning employing shorter ischemic periods (stunning models), or *3*) after hypothermic global ischemia, as evidenced previously by our laboratory (36).

Arrhythmias. The role of IP in minimizing ischemic-related arrhythmias remains controversial. In part, this may be attributed to species differences. For example, IP induces antiarrhythmic effects in rats (35, 41), whereas in swine, profibrillatory effects were observed (16, present study). Wang et al. (41) reported that the antiarrhythmic effects of IP could be mimicked by the administration of a specific κ-opioid receptor agonist, but not by δ-agonist DADLE in rats. Conversely, pharmacological preconditioning with δ_1-opioid agonist TAN-67 has also been shown to reduce arrhythmias in the rat model (14). In swine, only indirect evidence for a role of opioid agonists in arrhythmogenesis exists. Specifically, naloxone has been reported to decrease acute coronary occlusion-induced arrhythmic activity in anesthetized swine, theoretically suggesting proarrhythmic activity of opioids in this model (5). The infarct size-limiting effects of DPDPE or deltorphin-D were not associated with significant antiarrhythmic effects, as has been shown for TAN-67 in a rat model (14). However, none of the DPDPE- or deltorphin-D-preconditioned animals elicited lethal arrhythmias (intractable VF), which was highly significant compared with IP ($P < 0.01$) and

only marginally significant compared with controls ($P = 0.08$). Whereas these results clearly indicate a profibrillatory effect of IP, significant effects between DPDPE or deltorphin-D and controls may have been observed in studies with greater statistical power.

Interestingly, in the present study, DADLE was proarrhythmic during ischemia when compared with controls. Importantly, the coadministration of nor-BNI and DADLE completely abolished this effect. In further exploratory studies, we found that pentazocine, an opioid agonist primarily considered to act on the κ-receptors, induced invariably intractable VF shortly after LAD occlusion (5–10 min). Therefore, it can be speculated that κ-opioid receptor stimulation plays a role in arrhythmogenesis in swine. This hypothesis is being actively investigated in our laboratory.

Clinical outlook. When considering the pharmacological preconditioning benefits observed here, one needs to be careful about the opioid receptor type activated. For example, one important question is whether commonly clinically used opioid agonists such as morphine, fentanyl, etc., confer a similar myocardial protection as the agonists studied here relative to various clinical settings. Experimentally, preconditioning with morphine specifically reduced infarct size in rats (30) and rabbits (22). Whereas a role of the δ-opioid receptor stimulation in IP was described in humans (3, 37), to our knowledge no information exists on the specific infarct size-reducing effects of exogenously administered opioids in large mammalians. Yet, the commonly used opioid agonist fentanyl was not found to be cardioprotective in isolated rabbit hearts, as opposed to morphine, buprenorphine, and pentazocine (4). Importantly, in vivo, morphine was only protective at supraclinical dosages (3 mg/kg) in rabbits and not protective at 1 mg/kg in swine (unpublished observations from our laboratory). Nonspecific opioid agonists may also have low potency, thereby producing μ-opioid or κ-opioid receptor-related side effects at doses needed to produce cardioprotective effects via the δ-opioid receptor. Furthermore, by stimulating other receptor subtypes, nonspecific agonists may potentially cause an "antipreconditioned state" or less beneficial effects relative to those previously reported (1, 34, present study).

Importantly, many of these opioid agonists, including DPDPE, also have analgesic effects (2, 17), and potentially protect other vital organ systems from ischemic damage, such as the brain (9, 21, 24, 42). Taken together, these findings suggest that pharmacological preconditioning employing highly specific δ-agonists may be of great clinical importance. Such agents could be given before surgery in patients at high risk for an operative or postoperative ischemic event (e.g., off-bypass or on-bypass cardiac surgery, PTCA, and stenting procedures).

In conclusion, this study provides the first evidence that δ-opioid receptor stimulation is cardioprotective in the swine heart. Both the specific δ_1-opioid receptor agonist DPDPE and the novel δ_2-agonist deltorphin-D conferred infarct size-limiting effects. Another interesting finding of the current study was that δ-opioid agonist DADLE was not cardioprotective but even arrhythmogenic when given alone. When κ-opioid receptors were blocked with nor-BNI, DADLE not only conferred cardioprotection but its proarrhythmic effects were abolished, a finding that definitely deserves further study. However, no attenuation of acute stunning or ischemia-related arrhythmias were associated with δ-opioid-mediated cardioprotection in swine model of regional ischemia and reperfusion. Finally, this study suggests that specific δ-receptor opioid agonists may have clinical potential for cardioprotection in humans.

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