Consequences of opiate agonist and antagonist in myocardial ischaemia suggest a role of endogenous opioid peptides in ischaemic heart disease

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Objective: The aim was to evaluate the effects of an opiate agonist (U50,488H) and an opiate antagonist (naloxone) in myocardial ischaemia. **Methods:** A left thoracotomy was performed and the left coronary artery was ligated in adult Sprague-Dawley rats of either sex (350-400 g). Blood pressure, heart rate and electrocardiogram were measured before and after injections of U50,488H or naloxone and throughout the 30 min postligation period. **Results:** Following coronary artery occlusion, all rats in the control group developed arrhythmias, bradycardia, and hypotension. U50,488H potentiated and naloxone attenuated the ischaemia induced arrhythmias, bradycardia, and hypotension. **Conclusions:** The potentiating and blocking effects of U50,488H and naloxone, respectively, suggest that endogenous opioid peptides are involved in the pathophysiology of myocardial ischaemia and play an important role in ischaemic heart disease.

The endogenous opioid system includes three major families of peptides: dynorphins (derived from preproenkephalin B), endorphins (derived from prepro-opiomelanocortin), and enkephalins (derived from preproenkephalin A). Multiple forms of opioid peptides are derived from these major precursors and many of them possess potent cardiovascular properties.

Endogenous opioid peptides and opioid receptors are widely distributed throughout the body.[1] Since the identification of endogenous opioid peptides and opioid receptors in the heart,[2] it has become clear that the opioid system is involved in various cardiovascular stress situations such as shock,[3] heart failure,[4] and myocardial ischaemia.[5] In the isolated perfused rat heart, we have previously shown that endogenous opioid peptides caused cardiac arrhythmias[6] and that naloxone reversed the arrhythmias induced by myocardial ischaemia,[7] suggesting a role of these peptides in ischaemia induced arrhythmogenesis (for a review, see [8]). In the present in vivo study, if ischaemia induced arrhythmias were mediated through release of endogenous opioid peptides, then stimulation or blockade should also potentiate or attenuate such arrhythmias. We therefore investigated the effects of an opiate agonist (U50,488H) and an opiate antagonist (naloxone) following coronary artery ligation in the intact rat.

The κ receptor agonist dynorphin has been shown to be present in the heart by immunological techniques.[9] We used the κ receptor agonist U50,488H in this study because we have previously shown that it is more potent than dynorphin in the induction of ventricular arrhythmias which also result from myocardial ischaemia.[10 11] Naloxone was used in this study because it is a general opiate antagonist with μ and κ receptor blocking activity, which has been shown to abolish the arrhythmias induced by dynorphin or myocardial ischaemia.[10] The effects of U50,488H and naloxone in myocardial ischaemia as demonstrated in the present study indeed suggest that endogenous opioid peptides may play a role in the pathophysiology of myocardial ischaemia and the opiate antagonists may have potential therapeutic value in the prevention and treatment of ischaemic heart disease.

Methods

In all experiments, Sprague-Dawley rats of either sex weighing 350-400 g were used. All experiments were conducted according to the guidelines for animal experiments at Taichung Veterans General Hospital Medical Research Centre. The rats were anaesthetised with pentobarbitone sodium (60 $mg \cdot kg^{-1}$) intraperitoneally. A tracheotomy was performed and the intubated cannula was connected to a rodent ventilator (Harvard Apparatus, Massachusetts, USA). The animals were ventilated artificially with room air. Respiratory rate was synchronised with the rat's spontaneous rate (60-80 $strokes \cdot min^{-1}$, 1 $ml \cdot 100\ g^{-1}$). The left femoral artery and vein were cannulated for the measurement of blood pressure and heart rate by a Statham pressure transducer and a Biotechnometer (Gould), and for the administration of drugs, respectively. Electrocardiograms were recorded from lead II limb leads, with a positive electrode connected to the left hind leg, a negative electrode to the right foreleg, and a ground electrode to the left foreleg. For all recordings, Lifepak ECG Monitor (Physio-Control Corp, USA) was used.

After a left thoracotomy in the fifth intercostal space approximately 2 mm to the left of the sternum, the pericardium was opened and the heart exteriorised. A

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ligature (6/0 silk suture) was placed around the left coronary artery close to its origin and the heart was repositioned in the thoracic cavity. The rat was then allowed to stabilise for 15 min. Any rat showing functional instability such as hypotension or occurrence of cardiac arrhythmias was discarded. After equilibration, an opiate agonist U50,488H (0.92 μmol·kg^{-1}, Upjohn Co, USA), naloxone (2.75 μmol·kg^{-1}, Sigma, USA), or saline as control, were injected intravenously 10 min before the ligature was tied. Blood pressure, heart rate, and electrocardiogram were then continuously monitored throughout the 30 min postligation period.

To enable quantitative comparison, an arrhythmia scoring system modified from that of Curtis and Walker[12] was used. Each rat was given one score, representing the most severe type of arrhythmia observed during the entire postligation period. The details of the scoring system were: score 0=no arrhythmia; score 1=occasional ventricular premature contraction; score 2=frequent ventricular premature contractions; score 3=ventricular tachycardia, 1-2 episodes; score 4=ventricular tachycardia, 3-5 episodes; score 5=ventricular tachycardia, >5 episodes; score 6=ventricular fibrillation, 1-2 episodes; score 7=ventricular fibrillation, 3-5 episodes; score 8=ventricular fibrillation, >5 episodes.

Statistics

The χ^2 test was used to analyse the difference in the incidence of arrhythmias between control and treated groups, and between the groups receiving 2.75 μmol·kg^{-1} U50,488H with and without pretreatment with naloxone. Student's *t* test was used to test the difference in arrhythmia score and in the onset of arrhythmias between control and treated groups, and between the groups receiving 2.75 μmol·kg^{-1} U50,488H with and without pretreatment with naloxone, respectively. Analysis of variance was used to compare the difference in time course changes in mean arterial pressure and heart rate between control and treated groups, and between the groups receiving 2.75 μmol·kg^{-1} U50,488H with and without pretreatment of naloxone, respectively. A p value of less than 0.05 was considered as statistically significant. Values are given as means (SEM).

Results

In the doses used in this study, U50,488H and naloxone had no significant effects on the electrocardiogram, blood pressure, and heart rate prior to coronary artery occlusion. No arrhythmia was observed before or after administration of saline, U50,488H, or naloxone. The mean blood pressures in the control group before and after saline injection were 98.67 (SEM 3.92) and 98.67(3.22) mm Hg respectively, while the corresponding values in the group treated with U50,488H (2.75 μmol·kg^{-1}) were 96.9(7.68) and 98.86(8.41) mm Hg, and in the group treated with naloxone (2.75 μmol·kg^{-1}), 99.76(4.02) and 100(3.74) mm Hg. Similarly the heart rates in the control group before and after administration of saline were 394(31.83) and 386(10.80) beats·min^{-1} while the corresponding values in the group receiving U50,488H were 396(30.98) and 371(12.26) beats·min^{-1}, and in the group receiving naloxone, 390(9.96) and 406(10.91) beats·min^{-1}, respectively.

The table summarises the effects of U50,488H and naloxone on the cardiac rhythm following coronary artery occlusion. Myocardial ischaemia invariably caused ventricular arrhythmias, including ventricular premature contractions, ventricular tachycardia, and ventricular fibrillation. After coronary artery ligation, all rats in the control group developed ischaemia induced arrhythmias in the 30 min postligation period. Of seven rats, seven showed ventricular premature contractions and ventricular tachycardia and four showed ventricular fibrillation, with onset of arrhythmias at 2.34, 12, and 20 min, respectively. One rat died of ventricular fibrillation at 20 min, and the overall arrhythmia score was 5. Pretreatment of U50,488H before coronary artery ligation substantially potentiated the incidence and severity of the ischaemia induced arrhythmias. All rats developed ventricular premature contractions, ventricular tachycardia, and ventricular fibrillation, with onset of arrhythmias at 2.06, 4.86, and 6 min, respectively. Five out of seven rats died at 18 min, and the overall arrhythmia score was 7.57, which was significantly higher than that of the control ($p<0.01$). On the other hand, pretreatment with naloxone before coronary artery occlusion significantly reduced the incidence and severity of ischaemia induced arrhythmias. Of seven rats, seven showed ventricular premature contractions but only three developed ventricular tachycardia and only one developed ventricular fibrillation, with onset of arrhythmias at 12.57, 13.67, and 8.0 min, respectively. All these rats survived and the overall arrhythmia score was 2.29, which was significantly lower than in the control group ($p<0.05$).

Figures 1 and 2 show the effects of U50,488H and naloxone on the changes in mean arterial blood pressure and heart rate following coronary artery occlusion. Myocardial ischaemia invariably caused a marked decrease in both these variables. After coronary artery ligation, all rats in the control group had profound reduction in both mean arterial pressure and heart rate in the 30 min postligation period. Pretreatment with U50,488H led to a further reduction in both mean arterial pressure ($p<0.01$) and heart rate ($p<0.01$), and the values became significantly lower than in control. On the other hand, compared to the control group, pretreatment

Effects of U50,488H and naloxone on the cardiac rhythm following coronary artery occlusion in the rat. Values are means (SEM)

Group	*N*	*Arrhythmia score*	*VPC*		*VT*		*VF*		*Survival*
			n	*Onset (min)*	*n*	*Onset (min)*	*n*	*Onset (min)*	
Control	7	5(1.63)	7	2.34(2.93)	7	12(8.41)	4	20(7.62)	6
Naloxone (0.92 μmol·kg^{-1})	7	3(0.57)*	7	9.63(2.49)*	5	11(2.45)	2	8(2)	7
Naloxone (2.75 μmol·kg^{-1})	7	2.29(1.89)*	7	12.57(8.75)*	3†	13.67(10.69)	1	8	7
U50,488H (0.92 μmol·kg^{-1})	7	5.71(1.02)	7	10.57(2.91)*	5	8(1.81)	5	11.4(2.38)	4
U50,488H (2.75 μmol·kg^{-1})	7	7.57(0.53)**	7	2.06(2.53)	7	4.86(0.9)*	7	6(1.29)***	2†
Naloxone (0.92 μmol·kg^{-1}) +U50,488H (2.75 μmol·kg^{-1})	7	3(0.76)‡‡	7	6(0.87)‡	3§	12(2.27)‡	1§§§	9	7§§
Naloxone (2.75 μmol·kg^{-1}) +U50,488H (2.75 μmol·kg^{-1})	7	2(0.87)‡‡	4	10.75(4.52)‡	2§§	6.5(0.5)‡	1§§§	6	7§§

N and n are number of rats. VPC=ventricular premature contraction; VT=ventricular tachycardia; VF=ventricular fibrillation.
*, **, ***:$p<0.05$, <0.01, <0.001 *v* corresponding control group (Student's *t* test); †$p<0.05$ *v* corresponding control group by χ^2 test. ‡, ‡‡:$p<0.01$ and <0.001 *v* group receiving 2.75 μmol·kg^{-1} U50,488H by Student's *t* test; §, §§, §§§:$p<0.05$, <0.01, <0.001 *v* group receiving 2.75 μmol·kg^{-1} U50,488H by χ^2 test.

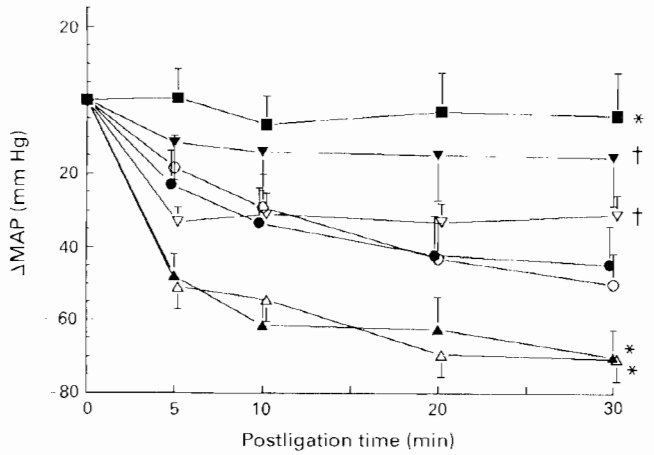

Figure 1 *Effects of U50,488H and naloxone on the change in mean arterial pressure (ΔMAP) following coronary artery occlusion in the rat. (○) saline; (●) naloxone 0.92 μmol·kg⁻¹; (■) naloxone 2.75 μmol·kg⁻¹; (△) U50,488H 0.92 μmol·kg⁻¹; (▲) U50,488H 2.75 μmol·kg⁻¹; (▽) naloxone 0.92 μmol·kg⁻¹+ U50,488H 2.75 μmol·kg⁻¹; (▼) naloxone 2.75 μmol·kg⁻¹+ U50,488H 2.75 μmol·kg⁻¹. Values are means, bars=SEM, of seven animals.*
$p<0.05$ v control by analysis of variance; †$p<0.05$ v group receiving 2.75 μmol·kg⁻¹ U50,488H by analysis of variance.

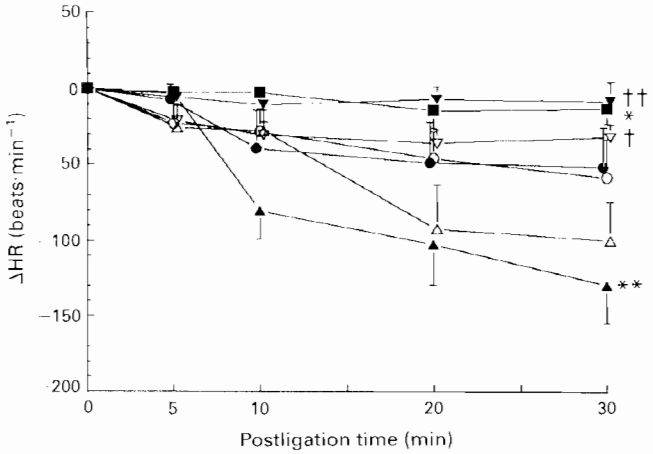

Figure 2 *Effects of U50,488H and naloxone on the change in heart rate (ΔHR) in beats·min⁻¹ following coronary artery occlusion in the rat. For key to symbols see fig 1 legend.*
*$p<0.05$; **$p<0.01$ v control; †$p<0.05$, ‡‡$p<0.01$ v 2.75 μmol·kg⁻¹ U50,488H .*

with naloxone reversed the reduction in both mean arterial pressure ($p<0.01$) and heart rate ($p<0.05$) due to coronary artery occlusion.

Pretreatment with naloxone followed by U50,488H and coronary artery ligation significantly attenuated the potentiating effects of U50,488H on ischaemia induced arrhythmias, hypotension, and bradycardia in a dose related manner, indicating that the potentiating effects of U50,488H and the blocking effects of naloxone against myocardial ischaemia were via the activation and blockade of the endogenous opioid system, respectively.

Discussion

It is well known that coronary artery occlusion leads to cardiogenic shock, bradycardia, and ischaemia induced arrhythmias, all of which are fatal complications secondary to acute myocardial infarction. Similar effects were shown in the present study in the rat, in which acute coronary artery ligation soon led to malignant arrhythmias, marked reduction in the arterial blood pressure, bradycardia, and even to death.

The potentiating effects on ischaemia induced arrhythmias, hypotension, and bradycardia of intravenous opiate agonist injections in the situation of acute coronary artery occlusion have so far not been reported. The findings of this study clearly show, for the first time, that the specific opiate agonist U50,488H not only increased the incidence and severity of ischaemia induced arrhythmias but also further decreased the arterial blood pressure and heart rate due to acute coronary artery occlusion. Such findings are compatible with our previous experiments in which U50,488H induced atrial and ventricular arrhythmias in the isolated perfused rat heart.[11] Results of this study also clearly show that naloxone blocks the arrhythmias, hypotension, and bradycardia secondary to myocardial ischaemia. Such findings are in agreement with our previous experiments on the ischaemic isolated rat heart in which pretreatment with naloxone markedly attenuated the cardiac arrhythmias and abolished the reduction in left ventricular pressures resulting from myocardial ischaemia and reperfusion.[7]

Direct actions of opioid peptides on the heart are supported by the existence of both opioid peptides and receptors in the heart. Lang *et al*[13] were able to show the presence of enkephalins in the guinea pig heart. Immunoreactive dynorphin[14] and prodynorphin derived opioid peptides[9] have also been shown to be present in the hearts of rats and guinea pigs, respectively. The existence of opioid receptors in the heart has been demonstrated by the blocking action of naloxone against cardiac effects of opioids in many studies. Receptor binding studies by Krumin *et al*[2] using μ, δ, and κ agonists to displace H-diplenorphin suggested the presence of μ and κ receptors in both atrium and ventricle. Results of the present study indicate that the κ receptor may be the likely receptor subtype that is involved in arrhythmogenesis during myocardial ischaemia. Previous studies showed that dynorphin,[10] another selective κ receptor agonist, induce ventricular arrhythmias, while β endorphin,[6] an agonist with high affinities to μ and δ receptors, causes atrial arrhythmias only in the isolated rat heart. Sitsapesan and Parratt[15] also found that in the anaesthetised rat MR2266, a selective κ receptor antagonist, is the most potent antiarrhythmic agent among the three types of opioid antagonists during ischaemia. Nevertheless, the notion that stimulation or blockade of endogenous opioid peptides potentiates or attenuates the ischaemia induced arrhythmias, bradycardia, and hypotension respectively is evidenced in this study, indicating that these peptides are involved in the pathophysiology of myocardial ischaemia.

In previous in vitro studies, we showed that endogenous opioid peptides are involved in ischaemia induced arrhythmogenesis. In the present in vivo study, the blocking effects of naloxone and the potentiating effects of U50,488H further infer that endogenous opioid peptides are factors in myocardial ischaemia. The results are also consistent with the hypothesis that these peptides may be released from the heart upon myocardial ischaemia, thus causing arrhythmias, hypotension, and bradycardia. By virtue of their antagonistic and agonistic action against opiates, naloxone and U50, 488H can rectify or potentiate these fatal complications secondary to myocardial ischaemia, thus suggesting an important role of endogenous opioid peptides in ischaemic

heart disease. The beneficial effects of opiate antagonism have considerable clinical implications in the prevention and treatment of ischaemic heart disease. Further studies are needed to define the therapeutic value of opiate antagonists.

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Key terms: endogenous opioid peptide; myocardial ischaemia

1 Hughes J, Kosterlitz HW, Smith TW. The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. *Br J Pharmacol* 1977;**61:**639–47.
2 Krumins SA, Faden AL, Feuerstein G. Opiate binding in rat hearts: modulation of binding after hemorrhagic shock. *Biophys Res Commun* 1985;**127:**120–8.
3 Holaday JW. Cardiovascular consequences of endogenous opiate antagonism. *Biochem Pharmacol* 1983;**32:**573–83.
4 Liang CS, Imai N, Stone CK, Woolf PD, Kawashima S, Tuttle RR. The role of endogenous opioids in congestive heart failure: effects of nalmefene on systemic and regional hemodynamics in dogs. *Circulation* 1987;**75:**443–51.
5 Fagbemi O, Lepran I, Parratt JR, Szekeres L. Naloxone inhibits early arrhythmias resulting from acute coronary ligation. *Br J Pharmacol* 1982;**76:**504–6.
6 Lee AYS, Zhan CY, Wong TM. Effects of β-endorphin on the contraction and electrical activity of the isolated perfused rat heart. *Int J Peptide Protein Res* 1984;**24:**525–8.
7 Zhan CY, Lee AYS, Wong TM. Naloxone blocks the cardiac effects of myocardial ischemia and reperfusion in the isolated rat heart. *Clin Exp Pharmacol Physiol* 1985;**12:**373–8.
8 Lee AYS. Endogenous opioid peptides and cardiac arrhythmias. *Int J Cardiol* 1990;**27:**145–51.
9 Weihe E, McKnight AT, Corbett AD, Kosterlitz HW. Proenkephalin- and prodynorphin-derived peptides in guinea-pig heart. *Neuropeptides* 1985;**5:**453–6.
10 Wong TM, Lee AYS. Chronic morphine treatment reduces the incidence of ventricular arrhythmias in the isolated rat heart induced by dynorphin or myocardial ischemia and reperfusion. *Neurosci Lett* 1987;**77:**61–5.
11 Wong TM, Lee AYS, Tai KK. Effects of drugs interacting with opioid receptors during normal perfusion or ischemia and reperfusion in the isolated rat heart – an attempt to identify cardiac opioid receptor subtypes involved in arrhythmogenesis. *J Mol Cell Cardiol* 1990;**22:**1167–75.
12 Curtis MJ, Walker MJA. Quantification of arrhythmias using scoring systems: an examination of seven scores in an in vivo model of regional myocardial ischemia. *Cardiovasc Res* 1988;**22:**656–65.
13 Lang RE, Hermann K, Dietz R, *et al*. Evidence for the presence of enkephalins in the heart. *Life Sci* 1983;**32:**399–406.
14 Spampinato S, Goldstein A. Immunoreactive dynorphin in rat tissue and plasma. *Neuropeptides* 1983;**3:**193–212.
15 Sitsapesan R, Parratt R. The effects of drugs interacting with opioid receptors on the early ventricular arrhythmias arising from myocardial ischemia. *Br J Pharmacol* 1989;**97:**795–800.