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Opioids in myocardial ischaemia: potentiating effects of dynorphin on ischaemic arrhythmia, bradycardia and cardiogenic shock following coronary artery occlusion in the rat

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The endogenous opioid peptide (EOP) dynorphin and opioid receptors have been found in the heart. This opioid system plays important roles in cardiovascular regulation and is involved in the pathophysiology of shock, heart failure and myocardial ischaemia. The aim of this study was to evaluate whether the EOP dynorphin modulates or potentiates ischaemia-induced arrhythmias and whether its effects are prevented by the opiate antagonist naloxone. Following coronary artery occlusion, all rats in the control group developed ischaemia-induced arrhythmias, bradycardia and hypotension, which were significantly potentiated by pre-treatment with dynorphin and attenuated by treatment with naloxone. The results clearly indicate that EOPs may be released when myocardial ischaemia occurs, thus causing arrhythmias, bradycardia and hypotension. Dynorphin and naloxone, by virtue of their opioid agonistic and antagonistic actions, respectively, potentiate and attenuate these fatal complications secondary to myocardial ischaemia. This suggests that EOPs play an important part in ischaemic heart disease.

Introduction

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

Endogenous opioid peptides (EOPs) and their receptors are present in brain areas responsible for cardiovascular control, in the heart, in autonomic ganglia and in adrenal medulla[1 3]. This widespread distribution and localization of EOPs throughout the cardiovascular system exerts biological activities which influence and regulate cardiovascular functions, both centrally and peripherally, including direct action of EOP on the heart. Exogenous EOPs have been found to induce different effects on blood pressure and heart rate depending on the route of administration[4,5]. EOPs may also act directly on the heart causing cardiac arrhythmias (for a review, see[6]). For instance, when administered directly into the heart, both beta-endorphin[7] and dynorphin[8] caused cardiac arrhythmias in the isolated perfused rat heart.

There is good evidence that EOPs may be released from the pituitary gland during various cardiovascular stress situations, such as shock[9] and myocardial ischaemia[10], which might contribute to their respective detrimental effects. For instance, the opioid antagonist naloxone has been shown to reverse hypotension in patients with cardiogenic or septic shock[11], and it has also been demonstrated that naloxone reversed the ischaemic arrhythmias that resulted from acute coronary artery occlusion and/or reperfusion in the rat and dog, both in in vitro and in vivo preparations[10,12,13]. According to Sawynok *et al.*[14], more compelling proof of the role of EOPs in any situation requires several lines of evidence, including the production of the effect by an opioid agonist and the opposite effect by an antagonist. The present study therefore aims at elucidating whether the EOP dynorphin and the opioid antagonist naloxone affect ischaemic arrhythmia, bradycardia and cardiogenic shock following myocardial ischaemia in the rat.

Immunological techniques have demonstrated the presence of dynorphin in the heart[15]. We used dynorphin in this study because we have previously shown that it is more potent than beta-endorphin or met-enkephalin in the induction of cardiac arrhythmias[7,8]. Naloxone was used because it is a general opiate antagonist which has been shown to abolish arrhythmias induced by dynorphin[8]. The potentiating effects of dynorphin and the attenuating effects of naloxone in myocardial ischaemia, as demonstrated in the present study, indeed provide more compelling evidence to support the notion that EOPs may play a role in the pathophysiology of myocardial ischaemia and that opiate antagonists may have potential therapeutic value in the prevention and treatment of ischaemic heart disease.

Methods

Sprague-Dawley rats of either sex weighing 350 to 400 g were used. They were anaesthetized with pentobarbitone

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sodium (60 mg . kg^{-1}) intraperitoneally. A tracheotomy was performed and the intubated cannula was connected to a rodent ventilator (Harvard Apparatus, Massachusetts, U.S.A.); the rats were ventilated artificially with room air (60–80 strokes . min^{-1}, 1 ml . 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, with a positive electrode connected to the left leg, a negative electrode to the right arm, and a ground electrode to the left arm. The Lifepak ECG Monitor (Physio-Control Corp., U.S.A.) was used for all electrocardiographic recordings.

Left thoracotomy in the fifth intercostal space was then performed. The heart was exposed, the pericardium opened, and a ligature (6/0 silk suture) was placed around the left coronary artery close to its origin. The rat was then allowed a 15 min waiting period in order for its cardiovascular condition to stabilize. During this period, any rat showing functional instability such as hypotension or occurrence of cardiac arrhythmias was removed. A total of six rats were excluded from subsequent experimentation because of occurrence of arrhythmias in two and hypotension (blood pressure less than 90/60 mmHg) in four animals. Forty-eight rats were finally included into the study after surgical stabilization. After equilibration, dynorphin (150 and 300 nmole . kg^{-1}, Sigma, U.S.A.), naloxone (1·38 and 2·75 μmole . kg^{-1}, Sigma, U.S.A.), or saline as control, were injected intravenously 10 min before the ligature was tied. Drug administration was blinded and randomized. Blood pressure, heart rate and electrocardiogram were then continuously monitored throughout the 30 min post-ligation period.

To enable quantitative comparison, an arrhythmia scoring system modified from that of Curtis and Walker[16] was used. They demonstrated that by assigning a score to the arrhythmias observed for an animal, it was possible to calculate mean and standard error for a group and thereby conduct parametric statistical testing. They further showed that when used in conjunction with raw arrhythmia data (incidence and onset of arrhythmias), arrhythmia scores facilitate the quantification of arrhythmias, and have precision and accuracy in the analysis of arrhythmias. In this study, both arrhythmia scores and raw arrhythmia data were presented. Each rat was given one score, representing the most severe type of arrhythmia observed during the entire post-ligation period. Details of the scoring system were: score 0 = no arrhythmia; score 1 = occasional ventricular premature contractions (VPC); score 2 = frequent VPC; score 3 = ventricular tachycardia (VT), 1–2 episodes; score 4 = VT, 3–5 episodes; score 5 = VT, > 5 episodes; score 6 = ventricular fibrillation (VF), 1–2 episodes; score 7 = VF, 3–5 episodes; score 8 = VF, > 5 episodes. VT was defined as a successive run of at least six VPC, of uniform QRS complex. If there were irregular waves of varying amplitude and shape, it was considered to be VF. When there were three or more VPC occurring within 1 min, it was considered to be frequent. When less than three VPC occurred in a minute, it was occasional.

The chi-squared test was used to analyse the difference in the incidence of arrhythmias between control and treated groups, and between the groups receiving 300 nmole . kg^{-1} dynorphin with and without pre-treatment with naloxone. Student's t-test was used to test the difference in arrhythmia scores and in the onset of arrhythmias between control and treated groups, and between the groups receiving 300 nmole . kg^{-1} dynorphin with and without pre-treatment 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 300 nmole . kg^{-1} dynorphin with and without pre-treatment of naloxone, respectively. A P value of less than 0·05 was considered as statistically significant. Values are given as means and SEM.

Results

In the doses used in this study, dynorphin 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, dynorphin, or naloxone. The mean blood pressures in the control group before and after saline injection were 98 ± 3 and 99 ± 3 mmHg respectively, while the corresponding values in the group treated with dynorphin (300 nmole . kg^{-1}) were 102 ± 2 and 99 ± 4 mmHg and in the group treated with naloxone (2·75 μmole . kg^{-1}), 99 ± 3 and 101 ± 4 mmHg. Similarly the heart rates in the control group before and after administration of saline were 384 ± 30 and 387 ± 23 beats . min^{-1}, in the group receiving dynorphin 389 ± 31 and 381 ± 24 beats . min^{-1}, and in the group receiving naloxone, 390 ± 11 and 396 ± 18 beats . $min^{\pm 1}$, respectively.

Table 1 summarizes the effects of dynorphin and naloxone on the cardiac rhythm following coronary artery occlusion. Myocardial ischaemia invariably caused ventricular arrhythmias, including VPC, VT and VF. Following coronary artery ligation, all rats in the control group developed ischaemic arrhythmias in the 30 min post-ligation period. Of eight rats, eight showed VPC, seven had VT and four developed VF. The onset of arrhythmias were 3·50, 5·71 and 7·75 min, respectively. The overall arrhythmia score was 5·38. Pre-treatment with dynorphin before coronary artery occlusion substantially potentiated the incidence and severity of ischaemic arrhythmias. Of eight rats receiving 150 nmole . kg^{-1} dynorphin, all developed VPC and VT, and six showed VF, with onset of arrhythmias at 3·00, 5·54 and 7·60 min, respectively. At a higher dose, 300 nmole . kg^{-1}, dynorphin further potentiated the ischaemic arrhythmias. Of eight rats, all showed VPC, VT and VF, with onset of arrhythmias at 4·00, 5·00 and 7·50 min, respectively. The overall arrhythmia score was 7·38, which was significantly higher than in the control group. The effects of dynorphin were dose related, and attenuated by pre-treatment with naloxone, as indicated by significant reductions in incidence of arrhythmias and arrhythmia score. On the other hand, pre-treatment with naloxone alone before coronary artery occlusion

Table 1 *Effects of dynorphin and naloxone on the cardiac rhythm following coronary artery occlusion in the rat*

	N	Arrhythmia score	VPC		VT		VF	
			n	Onset (min)	n	Onset (min)	n	Onset (min)
Control	8	5·38 ± 0·80	8	3·50 ± 0·91	7	5·71 ± 0·84	4	7·75 ± 1·03
Dynorphin (150 nmole . kg $^{-1}$)	8	6·00 ± 0·42	8	3·00 ± 1·02	8	5·54 ± 0·65	6	7·60 ± 0·67
Dynorphin (300 nmole . kg^{-1})	8	7·38 ± 0·32*	8	4·00 ± 0·46	8	5·00 ± 0·33	8†	7·50 ± 1·10
Naloxone (1·38 μmole . kg $^{-1}$)	8	2·91 ± 0·55*	8	9·34 ± 2·42*	5	10·67 ± 8·38	2	11·25 ± 2·94
Naloxone (2·75 μmole . kg $^{-1}$)	8	2·30 ± 1·89*	8	12·57 ± 8·75*	3†	13·67 ± 10·69	1	18·00
Naloxone (2·75 μmole . kg $^{-1}$) + dynorphin (300 nmole . kg $^{-1}$)	8	4·00 ± 1·16‡	7	5·71 ± 1·34	5	10·00 ± 2·35	3§	14·67 ± 1·76‡

Values are mean ± SEM. N and n are number of rats. VPC = ventricular premature contraction; VT = ventricular tachycardia; VF = ventricular fibrillation.

*,†Statistically significant difference with the corresponding control group at the level $P < 0·05$, by Student's t-test and chi-squared test, respectively.

‡,§Statistically significant difference with the group receiving 300 nmole . kg $^{-1}$ dynorphin at the level $P < 0·05$ by Student's t-test, and at the level $P < 0·01$ by chi-squared test, respectively.

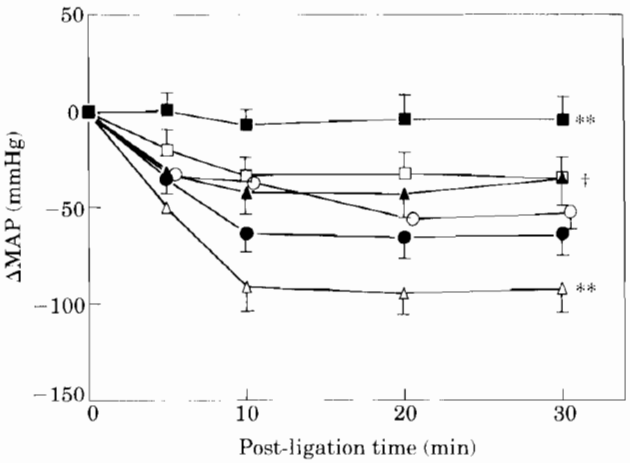

Figure 1 Effects of dynorphin and naloxone on the change in mean arterial pressure (ΔMAP) in mmHg following coronary artery occlusion in the rat. ○ = saline; ● = dynorphin 150 nmole . kg $^{-1}$; △ = dynorphin 300 nmole . kg $^{-1}$; □ = naloxone 1·38 μmole . kg $^{-1}$; ■ = naloxone 2·75 μmole . kg $^{-1}$; ▲ = naloxone 2·75 μmole . kg $^{-1}$ + dynorphin 300 nmole . kg $^{-1}$. Values are means and SEM of eight animals. $^{**}P < 0·01$ vs control by analysis of variance; †$P < 0·01$ vs group receiving 300 nmole . kg $^{-1}$ dynorphin by analysis of variance.

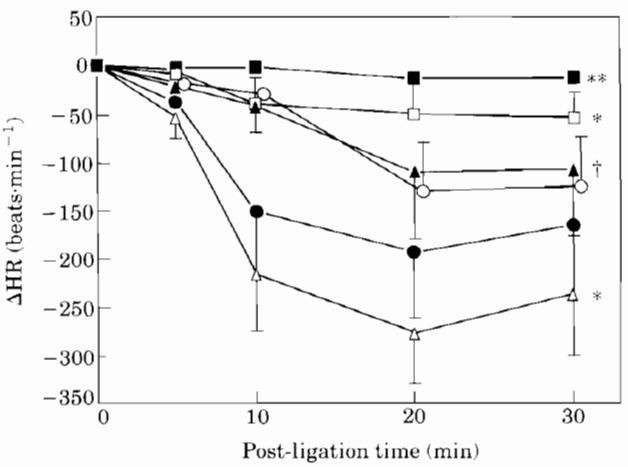

Figure 2 Effects of dynorphin and naloxone on the change in heart rate (ΔHR) in beats . min $^{-1}$ following coronary artery occlusion in the rat. For key to symbols see Fig. 1. *$P < 0·05$, **$P < 0·01$ vs control; †$P < 0·05$ vs 300 nmole . kg $^{-1}$ dynorphin.

significantly reduced the incidence and severity of ischaemic arrhythmias. Of eight rats receiving 2·75 μmole . kg $^{-1}$ naloxone, eight showed VPC but only three developed VT and only one developed VF, with onset of arrhythmias at 12·57, 13·67 and 18·00 min, respectively. The overall arrhythmia score was 2·30, which was significantly lower than in the control group.

Figs 1 and 2 show the effects of dynorphin 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 blood pressure and heart rate. Following coronary artery ligation, there were profound reductions in both mean arterial pressure and heart rate in the 30 min post-ligation period. Pre-treatment with dynorphin before coronary artery occlusion substantially potentiated the ischaemia-induced hypotension and bradycardia, leading to further reductions in both blood pressure and heart rate, and the values became significantly lower than in control. Moreover, pre-treatment with naloxone followed by dynorphin and coronary artery ligation significantly attenuated the potentiating effects of dynorphin on the ischaemia-induced hypotension and bradycardia. On the other hand, compared to the control group, pre-treatment with naloxone alone reversed the reduction in both mean arterial pressure and heart rate due to coronary artery occlusion.

The potentiating effects of dynorphin and the blocking effects of naloxone against ischaemia-induced arrhythmias, hypotension and bradycardia, strongly indicated that the effects of the EOP dynorphin in myocardial

ischaemia were via the activation of the endogenous opioid system.

Discussion

It is well-known that myocardial ischaemia may lead to cardiogenic shock, bradycardia, and ischaemic arrhythmias, all of which may be fatal complications secondary to acute myocardial infarction. In this study we have shown that acute coronary artery occlusion soon led to marked reductions in arterial blood pressure, bradycardia and malignant arrhythmias.

To support the involvement of EOPs in a particular physiological process, a number of criteria have been suggested[14]. These include the demonstration of, first, cross-tolerance with morphine; second, similar responses with other opiate antagonists; third, the lack of an effect with non-antagonist isomers; fourth, agents that agonize or antagonize EOPs, potentiating or attenuating the response, respectively, and fifth, a direct release of EOPs. There are several pieces of evidence suggesting that EOPs are involved in myocardial ischaemia. Firstly, isolated perfused hearts of chronically morphine-treated rats exhibited tolerance to dynorphin or ischaemia and reperfusion at induction of arrhythmias[17]. Secondly, opioid antagonists, naloxone[12], naltrexone[18] or MR2266[19] have been shown to attenuate arrhythmias during ischaemia and reperfusion in the isolated rat heart. Thirdly, the antiarrhythmic effect of naloxone is stereospecific, because naloxone (+) isomer (without opiate antagonistic property) is not effective in reversing the ischaemia-induced arrhythmias, hypotension and bradycardia in the rat[20]. Fourthly, as in this study, if ischaemia-induced arrhythmias are mediated through release of EOPs, then stimulation or blockade of EOPs should also potentiate or attenuate such arrhythmias, respectively. Our findings clearly show that dynorphin not only increased the incidence and severity of ischaemia-induced arrhythmias, but also further decreased the blood pressure and heart rate due to acute coronary artery occlusion. Results of the present study also clearly show that naloxone limited and delayed the occurrence of arrhythmias, hypotension, and bradycardia secondary to myocardial ischaemia. Such findings are compatible with our previous experiments in which dynorphin induced cardiac arrhythmias[8], and naloxone attenuated the arrhythmias and abolished the reduction in left ventricular pressures resulting from myocardial ischaemia and reperfusion in the rat isolated heart[12]. The above strongly indicate that EOPs are involved in the pathophysiology of myocardial ischaemia. The most important questions outstanding, therefore, are the demonstration of a direct release of EOPs, the identification of the EOPs that are released, the EOP levels before and after myocardial ischaemia, and the elucidation of the events that occur following myocardial ischaemia, which await further study.

It is known that acute myocardial ischaemia invariably exhibits increased activity of the autonomic nervous system. Thus bradycardia and hypotension reflect augmented vagal activity, whereas ischaemic arrhythmias denote the enhanced sympathetic activity which promotes the development of arrhythmias. The cause of the vagotonia and resultant bradycardia and hypotension accompanying myocardial ischaemia is not entirely clear. It may be due to the Bezold-Jarisch reflex or vasodepressor response. Moreover the significance of bradycardia is debated. There is evidence, on the one hand, that bradycardia during acute myocardial ischaemia enhances the development of ventricular arrhythmias and hypotension[21]. On the other hand, it has been suggested that increased vagal tone that produces bradycardia during the early phase of myocardial ischaemia may actually be protective, perhaps because it reduces myocardial oxygen demands[22]. Nevertheless, during myocardial ischaemia, the mechanism of the endogenous opioid system in the modulation of blood pressure, heart rate, arrhythmias, and their interrelationship remains to be elucidated.

In previous in vitro studies, we showed that EOPs are involved in ischaemia-induced arrhythmogenesis. In the present in-vivo study, the potentiating effects of dynorphin and the blocking effects of naloxone further infer that EOPs 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 respective agonistic and antagonistic actions against opiates, dynorphin and naloxone can potentiate or attenuate these fatal complications secondary to myocardial ischaemia, thus suggesting that EOPs play an important role 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 determine whether myocardial ischaemia causes release of EOP, and to define the therapeutic value of opiate antagonists.

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