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NALOXONE BLOCKS THE CARDIAC EFFECTS OF MYOCARDIAL ISCHAEMIA AND REPERFUSION IN THE RAT ISOLATED HEART

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SUMMARY

1. The effects of naloxone on contractility and cardiac rhythm were studied in the rat isolated perfused heart during myocardial ischaemia and reperfusion.

2. Pretreatment of the rat isolated perfused heart with naloxone abolished the reduction in left ventricular pressures and attenuated greatly the arrhythmias due to myocardial ischaemia and reperfusion.

3. Administration of naloxone into the fibrillating rat isolated heart induced by myocardial ischaemia and reperfusion also attenuated the arrhythmias in a dose-dependent manner.

4. The results indicate a possible involvement of the endogenous opioid peptides in the cardiac effects due to myocardial ischaemia and reperfusion. The anti-arrhythmic effect of naloxone has great clinical implications.

Key words: arrhythmia, left ventricular pressures, myocardial ischaemia, naloxone, rat isolated perfused heart, reperfusion.

INTRODUCTION

It has been shown that naloxone attenuates markedly the arrhythmias resulting from occlusion of the coronary artery in conscious and anaesthetized rats (Fagbemi *et al.* 1982), suggesting that endogenous opioid peptides are involved in the arrhythmogenesis induced by myocardial ischaemia. We have also found that naloxone reduces the incidence of fibrillation induced by administration of theophylline and inhalation of chloroform in the rat (Wong & Lee 1985). However, these *in vivo* studies do not provide information on whether naloxone acts on the brain or directly on the heart to produce this effect. It has also been shown that met-enkephalin is present in the heart (Lang *et al.* 1983; Weihe *et al.* 1983), suggesting a possible role of this endogenous opioid peptide in intracardiac regulation. This is supported by our findings in the rat isolated heart that β-endorphin causes arrhythmias and decreases contractility of the heart and that these effects are antagonized by naloxone (Lee *et al.* 1984). The *in vivo* antiarrhythmic effect of naloxone and the arrhythmogenic effect of β-endorphin in the isolated perfused heart suggest that the endogenous opioid peptides may be released from the heart upon myocardial ischaemia thus causing

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arrhythmias, and naloxone, by virtue of its antagonistic action against opiates, rectifies this irregular cardiac rhythm. In this study, using the rat isolated perfused heart preparation, we induced arrhythmias by myocardial ischaemia and reperfusion using a modified procedure described by Penny and Sheridan (1983) and studied the cardiac effects of naloxone in an effort to verify the above suggestion.

METHODS

Perfusion of isolated heart

Female Sprague-Dawley rats weighing 210–230 g were killed by decapitation. The heart was rapidly excised and mounted for perfusion by the Langendorff technique within 1 min. The hearts were perfused retrogradely, at a constant perfusing pressure of 70 mmHg and a flow rate of 3–4 ml/min, with oxygenated Kreb's ringer (pH = 7.4) containing (in mmol/l) NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.1, $NaHCO_3$ 24, $CaCl_2$ 2.5 and glucose 10, warmed by a water bath at a constant temperature of 35°C and equilibrated with a 95% O_2:CO_2 mixture throughout the experiment. A water jacket 1.0 cm thick, 9.0 cm long and with an inside diameter of 4.0 cm was used to provide an environment of constant temperature at around 32°C so that the temperature drop during myocardial ischaemia was within 1°C. Preliminary studies in our laboratory showed that a change of 1°C in temperature does not affect either the cardiac rhythm or contractility.

Measurements of left ventricular pressures and arrhythmias

A 24 gauge needle was inserted through the apex of the heart into the left ventricle for the measurement of left ventricular systolic and diastolic pressures, which were recorded on a polygraph by a Gould 2200S recorder via a pressure transducer (Statham). Electrocardiograms were recorded by a Heart Monitor System Model No. 633 BM (Fukuda, Japan), with a positive electrode hooked at the apex of the heart, a negative electrode at the atrium and a ground electrode at the pericardial tissues. A microsyringe (Hamilton) was positioned via the aortic cannula for the injection of naloxone.

Drugs

Naloxone from Dupont Pharmaceutical Co. was dissolved in Kreb's solution. A fixed volume of 20 μl of the drug or ringer was injected into the preparation in 1 min.

Treatments

Two series of experiments were performed. In the first series of experiments, naloxone was administered before ischaemia. The heart was allowed to equilibrate for 20–30 min after it had been mounted. Any heart showing functional instability in this period was discarded. Two minutes after administration of naloxone (100 μg), perfusion was stopped for 20 min after which the heart was reperfused. Preliminary experiments showed that naloxone did not cause any change in the left ventricular pressures, and electrical activities (Lee *et al.* 1984). Both left ventricular pressures and electrocardiogram (ECG) were recorded in the experiment. In the second series of experiments the isolated heart was induced to fibrillate by myocardial ischaemia, a procedure developed in our laboratory (Lee *et al.* 1985). Naloxone was administered after the appearance of ventricular fibrillation induced by myocardial ischaemia and reperfusion. Immediately after the

heart had been mounted, perfusion was stopped for 10 min and then reperfused. In this procedure there was no perfusion before ischaemia and about 80% of the hearts exhibited ventricular fibrillation (VF) 2–15 min after reperfusion. Naloxone at doses of 200 μg or 600 μg was administered when VF occurred. The ECG was recorded for 40 min after administration of naloxone. Preliminary experiments showed that fibrillating hearts would never recover after 40 min.

Statistical analyses

Values in the experiment on the left ventricular pressures were changes from the pre-ischaemic levels. Analysis of variance for split plot design and Chi square test were used to test differences between control and naloxone-treated groups in changes in left ventricular pressures and incidence of arrhythmias, respectively.

RESULTS

Effects of pretreatment with naloxone on left ventricular pressures

There was a marked reduction in both left ventricular diastolic and systolic pressures during myocardial ischaemia. The reduction seemed to be less in the hearts pretreated with naloxone (100 μg), but the difference was not statistically significant. Upon reperfusion the left ventricular pressures in the control group went up again, but not to the original levels (Fig. 1). In the naloxone pretreated group, the left ventricular diastolic and systolic pressures returned to above or back to the original levels, respectively (Fig. 1). They were significantly higher than the corresponding pressures in the control group.

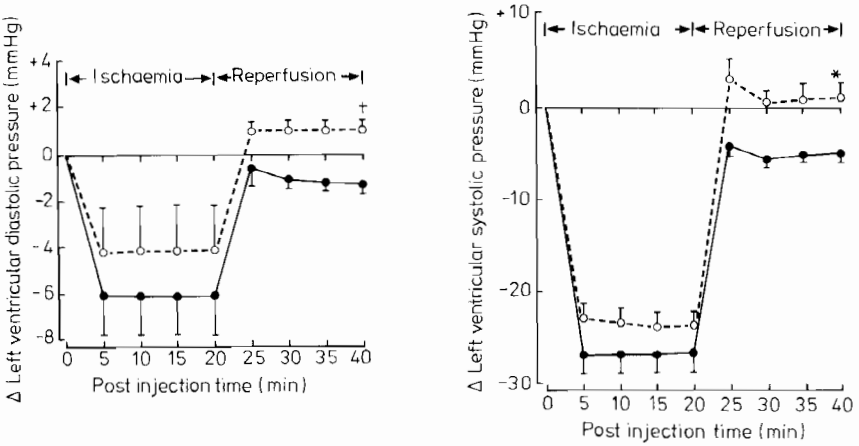

Fig. 1. Changes in left ventricular pressure in the rat isolated perfused heart during myocardial ischaemia and reperfusion—effects of pretreatment with naloxone (○). Values are changes in pressures (mmHg) during myocardial ischaemia and reperfusion and are presented as mean and s.e.m. The left ventricular diastolic pressures before treatment were 15 (s.e.m. = 1.12, $n = 10$) and 15 (s.e.m. = 1.27, $n = 10$) for the control and naloxone pretreated groups, respectively. The left ventricular systolic pressures before treatment were 38 (s.e.m. = 2.20, $n = 10$) and 37 (s.e.m. = 1.83, $n = 10$) for the same groups, respectively. Significant difference from the corresponding control groups during the reperfusion period to the levels * $P < 0.05$ and † $P < 0.01$, respectively using analysis of variance for split plot design.

Effects of pretreatment with naloxone on the cardiac rhythm

In the control group most hearts exhibited either atrial or ventricular arrhythmias or both. There were eight and three hearts showing atrial and ventricular arrhythmias during myocardial ischaemia, respectively whereas ten and eight hearts showed the same irregular cardiac rhythms during the reperfusion period (Table 1). In the naloxone-pretreated group, both the number of hearts showing arrhythmias and incidence of arrhythmias during myocardial ischaemia and reperfusion were significantly reduced except the number of hearts exhibiting atrial arrhythmias (Table 1). In addition, the life-threatening ventricular tachycardia and fibrillation occurred only in the control group but not in the naloxone-pretreated group (Table 1). The results indicate an attenuation of arrhythmias in the naloxone pretreated hearts.

Table 1. Incidence of cardiac arrhythmias in the rat isolated perfused heart during myocardial ischaemia and reperfusion—effect of pretreatment with naloxone

Group	Number of heart	Incidence of atrial arrhythmias		Incidence of ventricular arrhythmias	
		Myocardial ischaemia	Reperfusion	Myocardial ischaemia	Reperfusion
Control	10	8	10	3	8
Naloxone (100 μg)	10	3*	4†	0	0‡

Significant difference to the corresponding control groups at the levels * $P<0.05$, † $P<0.01$ and ‡ $P<0.001$, respectively by Chi square test. Atrial arrhythmias include second and third degree atrial-ventricular block, premature atrial contraction, paroxysmal atrial tachycardia and atrial fibrillation. Ventricular arrhythmias include premature ventricular contraction, ventricular tachycardia and ventricular fibrillation.

Effects of naloxone on the cardiac rhythm of fibrillating isolated perfused heart

In the control group, seven out of ten hearts did not recover at all after the appearance of VF and of the three hearts that recovered, two had abnormal cardiac rhythm; one having ventricular premature contraction (VPC) and the other both VPC and atrial-ventricular block (AV block). There was only one heart that recovered with a normal ECG within 40 min (Table 2). In the groups receiving 200 μg and 600 μg of naloxone, seven and nine hearts, respectively, out of ten recovered. In the group receiving 200 μg of naloxone, all three hearts that did not recover showed transient improvement which lasted for less than 3 min, indicating an unsuccessful attempt to recover. None of the seven hearts in the control group that did not recover showed any similar phenomenon. In addition, in the group receiving 600 μg of naloxone six hearts recovered within 20 min whereas only one heart in the control group recovered within the same time period (Table 2). The results indicate that naloxone helped the heart to recover from fibrillation and shortened the time required for recovery.

Table 2. Effects of naloxone on the cardiac rhythm in the ischaemia-induced fibrillating rat isolated heart

Group	Number of hearts	No recovery from fibrillation			Recovery from fibrillation (within min)			
		No improvement	Transient (<3 min) improvement	Total	20	30	40	Total
Control	10	7	0	7	1	1	1	3
Naloxone (200 μg)	10	0†	3	3	4	0	3	7
Naloxone (600 μg)	10	0†	1	1*	6	3	0	9*

Significant difference to the corresponding control groups at the levels * $P<0.01$ and † $P<0.001$, respectively by Chi-square Test.

DISCUSSION

The isolated heart preparation was used as a model for studying ventricular arrhythmia during myocardial ischaemia and reperfusion (Penny & Sheridan 1983). In this study we made use of this model to study the effect of naloxone on the contractility and cardiac rhythm of rat heart during myocardial ischaemia and reperfusion. As expected, we observed cardiac arrhythmias during myocardial ischaemia and reperfusion in the rat isolated heart as did Penny and Sheridan (1983) in guinea-pig heart. In agreement with their finding, the ventricular arrhythmias were more frequent and severe during reperfusion than during myocardial ischaemia (Table 1). Based on this, one of the two experimental designs used in this study was concentrated on the ventricular arrhythmias induced by myocardial ischaemia and reperfusion using a procedure developed in our laboratory.

In this study we found that pretreatment with naloxone attenuated markedly the cardiac arrhythmias resulting from myocardial ischaemia and reperfusion (Table 1). Administration of naloxone after the appearance of fibrillation induced by myocardial ischaemia and reperfusion also helped the heart to correct this life-threatening irregular cardiac rhythm (Table 2). The results support the antiarrhythmic effect of naloxone in the rat isolated atria (Eiden & Ruth 1982). The results also support the notion that endogenous opioid peptides may be involved in arrhythmogenesis of the heart.

It has been suggested that sudden marked coronary obstruction of coronary blood flow as well as reperfusion of a previously occluded artery may be responsible for the appearance of ventricular fibrillation (Abrahamsson *et al.* 1983). The blocking effect of naloxone on ventricular arrhythmias certainly has great clinical implications in the prevention and treatment of cardiac arrhythmias associated with ischaemic heart disease, Prinzmetal's syndrome, post-infarction syndrome and coronary by-pass surgery. Further studies are needed to define the therapeutic values of naloxone.

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