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Stereospecific antiarrhythmic effects of naloxone against myocardial ischaemia and reperfusion in the dog

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1 The effects of both the (−)- and (+)-stereoisomers of naloxone in anaesthetized dogs with arrhythmias induced by acute coronary artery occlusion followed by reperfusion were investigated.

2 Following coronary artery occlusion and reperfusion, all dogs in the control group developed ischaemia- and reperfusion-induced cardiac arrhythmias, bradycardia and hypotension.

3 The opiate antagonist (−)-naloxone prevented the arrhythmias, bradycardia and hypotension due to myocardial ischaemia and reperfusion.

4 The (+)-stereoisomer of naloxone, which is inactive as an opiate antagonist, was without beneficial effects.

5 These results indicate a possible involvement of endogenous opioid peptides in the cardiac effects due to myocardial ischaemia and reperfusion, mediated by opiate receptors through opiate antagonism.

Keywords: Endogenous opioid peptide; naloxone; stereoisomer; cardiac arrhythmias; myocardial ischaemia and reperfusion

Introduction

It has been shown that endogenous opioid peptides (EOP) are involved in cardiac arrhythmogenesis (for a review, see Lee, 1990) and that the opiate antagonists naturally possess antiarrhythmic activity (for a review, see Lee, 1989). The pure opiate antagonist, naloxone, has been found to inhibit cardiac arrhythmias resulting from coronary artery occlusion in rats (Fagbemi *et al.*, 1982; Lee *et al.*, 1992) and dogs (Huang *et al.*, 1986), suggesting that EOP may be released from the heart upon myocardial ischaemia thus causing arrhythmias, and naloxone, by virtue of its antagonistic action against opiates, rectifies this irregular cardiac rhythm. Further studies demonstrated that the antiarrhythmic effects of opiate antagonists (Mr 1452 and WIN 44,441-3) against myocardial ischaemia were stereospecific and thus mediated by opiate receptors since their (+)-isomers, which possess no opiate antagonistic properties, were less effective (Parratt & Sitsapesan, 1986). On the other hand, it has also been reported that both (−)- and (+)-stereoisomers of naloxone were antiarrhythmic in rats subjected to intracarotid administrations of adrenaline, suggesting that the antiarrhythmic action of naloxone was probably not mediated by opiate receptors (Sarne *et al.*, 1988). To clarify the discrepancy between the results obtained from the above two experimental models (ischaemia- or adrenaline-induced arrhythmias), and to provide more compelling evidence that EOP are indeed involved in the pathophysiology of myocardial ischaemia and reperfusion, the effects of both (−)- and (+)-stereoisomers of naloxone were investigated in anaesthetized dogs with arrhythmias induced by acute coronary artery ligation followed by reperfusion.

Methods

Mongrel dogs of either sex weighing between 10 to 20 kg were used. All experiments were conducted according to the guidelines for animal experiments at Taichung Veterans General Hospital Medical Research Centre. The animals were anaesthetized with pentobarbitone sodium (25 mg kg^{-1}) administered intravenously into the lateral saphenous vein. They were intubated and artificially ventilated. Respiratory rate was synchronized with that of the dog (16–18 strokes min^{-1}; 300 ml kg^{-1} min^{-1}). The left femoral artery and vein were cannulated for the measurement of blood pressure (BP) and heart rate (HR) with a Statham pressure transducer and a Biotechnometer (Gould), and for the administration of drugs, respectively. Electrocardiograms (ECG) were recorded from lead II limb leads, using the Lifepak ECG Monitor (Physio-Control., USA).

A similar procedure for coronary artery ligation in the dog to that described by Benfey *et al.* (1984) was adopted. Median thoracotomy was performed. The heart was exposed by cutting open the pericardium. The left anterior descending coronary artery (LAD) was isolated for ligation. A silk suture with a short polyethylene tube threaded around it was placed under the LAD. The dog was then allowed to equilibrate for 20 min. Afterwards, (−)-naloxone (with opiate antagonistic properties), (+)-naloxone (without opiate antagonistic properties), or 0.9% NaCl solution (as control) were infused into the femoral vein over a period of 10 min. At 2 min after the start of infusion, the LAD was occluded by applying tension on the suture and clamping immediately above the polyethylene tubing surrounding the artery. Occlusion was maintained for 20 min followed by reperfusion by simply releasing the clamp for 30 min.

Doses of (−)-naloxone used were 0.92 and 2.75 μmol kg^{-1}, and that of (+)-naloxone was 2.75 μmol kg^{-1}. They were dissolved in 5 ml of 0.9% NaCl solution. ECG, BP and HR were continuously monitored throughout the experiment. Arrhythmias were assessed by recording the incidence and onset of ventricular premature contraction (VPC), ventricular tachycardia (VT) and ventricular fibrillation (VF). A Chi-squared test was used to analyse the difference in the incidence of arrhythmias between control and drug-treated groups. Student's *t* test was used to test the difference in the onset of arrhythmias between control and drug-treated groups. 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. A P value of less than 0.05 was considered as statistically significant.

Results

Effects of stereoisomers of naloxone on cardiac arrhythmias

Table 1 summarizes the effects of the (−)- and (+)-stereoisomers of naloxone on cardiac rhythm following coronary artery occlusion and reperfusion in the dog. Myocar-

Table 1 Effects of stereoisomers of naloxone on cardiac arrhythmias during coronary occlusion and reperfusion

	Occlusion (20 min)							*Reperfusion* (30 min)					
			VPC		*VT*		*VF*		*VPC*		*VT*		*VF*
			onset		onset		onset		onset		onset		onset
	N	*n*	(min)	*n*	(min)	*n*	(min)	*n*	(min)	*n*	(min)	*n*	(min)
Control	8	8	1.69 ± 0.42	4	6.50 ± 3.84	3	9.00 ± 5.00	8	1.69 ± 0.53	4	1.13 ± 0.31	5	1.20 ± 0.46
(−)-Naloxone (0.92 μmol kg^{-1})	8	7	3.57 ± 0.72*	1	2.00	1	4.00	5	2.60 ± 0.81	1	1.00	2	3.00 ± 1.00
(−)-Naloxone (2.75 μmol kg^{-1})	8	7	7.00 ± 2.43	0*		0		6	2.50 ± 0.56	0*		0*	
(+)-Naloxone (2.75 μmol kg^{-1})	8	8	1.13 ± 0.21	4	4.25 ± 0.85	1	9.00	8	1.31 ± 0.33	3	1.33 ± 0.33	3	1.88 ± 1.05

N and *n* represent the number of animals; VPC – ventricular permature contraction; VT – ventricular tachycardia; VF – ventricular fibrillation.
Statistical difference from the corresponding control values at the levels of $*P<0.05$ by chi-squared test, and $*P<0.05$ by Student's *t* test.

dial ischaemia and reperfusion invariably caused malignant ventricular arrhythmias including VPC, VT and VF. In agreement with previous findings (Penny & Sheridan, 1983; Zhan *et al.*, 1986), arrhythmias were more frequent and severe during the reperfusion period. Of 8 dogs in the control group, 8 showed VPC, 4 VT and 3 VF during ischaemia and 8 showed VPC, 4 VT and 5 VF during the reperfusion period. The onset of arrhythmias for VPC, VT and VF were 1.69, 6.50 and 9.00 min during ischaemia and 1.69, 1.13 and 1.20 min during the reperfusion periods, respectively. Pretreatment with (−)-naloxone significantly reduced the incidence and delayed the onset of arrhythmias in both the ischaemia and reperfusion periods in a dose-related manner. Of 8 dogs receiving 0.92 μmol kg^{-1} (−)-naloxone, 7 showed VPC, 1 VT and 1 VF during ischaemia and 5 showed VPC, 1 VT and 2 VF during the reperfusion period. The onset of arrhythmias for VPC, VT and VF were 3.57, 2.00 and 4.00 min during ischaemia and 2.60, 1.00 and 3.00 min during the reperfusion periods, respectively. Moreover, of 8 dogs receiving 2.75 μmol kg^{-1} (−)-naloxone, 7 showed VPC during ischaemia and 6 showed VPC during the reperfusion periods whilst no dog developed VT or VF. The onset of arrhythmias for VPC were 7.00 min during ischaemia and 2.5 min during reperfusion, respectively. Pretreatment with (+)-naloxone (2.75 μmol kg^{-1}), however, was without beneficial effects in preventing the ischaemia- or reperfusion-induced arrhythmias. Of 8 dogs, 8 showed VPC, 4 VT and 1 VF during ischaemia and 8 showed VPC, 3 VT and 3 VF during the reperfusion periods. The onset of arrhythmias for VPC, VT and VF were 1.13, 4.25 and 9.00 min during ischaemia and 1.31, 1.33 and 1.88 min during reperfusion, respectively. These differences were not statistically significant compared to the control group.

Effects of stereoisomers of naloxone on blood pressure and heart rate

In the doses used in this study, both stereoisomers of naloxone had no significant effects on BP or HR. The BPs in the control group before and after injection of saline were 99 ± 7 and 98 ± 8 mmHg respectively. while the corresponding values in the groups treated with 2.7 μmol kg^{-1} of (−)-naloxone were 93 ± 9 and 98 ± 9 mmHg, and with (+)-naloxone were 99 ± 7 and 102 ± 6 mmHg. Similarly the HRs in the control group before and after administration of saline were 189 ± 12 and 188 ± 13 beats min^{-1} respectively, whereas the corresponding values in the groups treated with 2.75 μmol kg^{-1} (−)-naloxone were 189 ± 2 and 193 ± 9 beats min^{-1}, and with (+)-naloxone were 186 ± 9 and 185 ± 8 beats min^{-1}.

The effects of the (−)- and (+)-stereoisomers of naloxone on the BP and HR following coronary artery occlusion and reperfusion in the dog are shown in Figures 1 and 2. Myocardial ischaemia and reperfusion invariably caused a marked decrease in both BP and HR. Pretreatment (−)-naloxone significantly prevented the reduction in BP HR during both the ischaemia and reperfusion periods dose-related manner. Pretreatment with (+)-naloxone, ever, was without beneficial effects in reversing the ischa or reperfusion-induced hypotension and bradycardia.

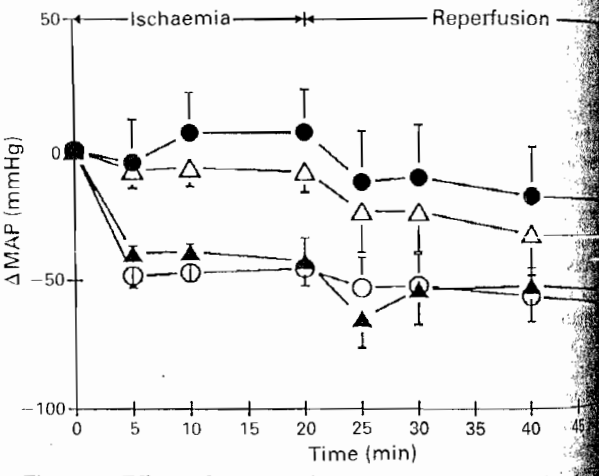

Figure 1 Effects of the stereoisomers of naloxone on the cha mean arterial pressure (Δ MAP) following coronary artery occ and reperfusion in the dog: (○) saline; (△) (−)-na 0.92 μmol kg^{-1}; (●) (−)-naloxone 2.75 μmol kg^{-1}; (▲) naloxone 2.75 μmol kg^{-1}. Values are means and s.e.mean (V bars) of eight animals. $*P<0.05$ vs control by analysis of va

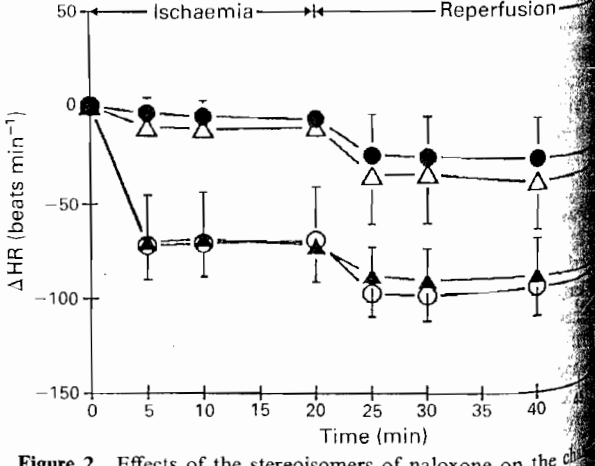

Figure 2 Effects of the stereoisomers of naloxone on the ch heart rate (Δ HR) in beats min^{-1} following coronary arte lusion and reperfusion in the dog. For key to symbols see fig Figure 1.

Discussion

It is well-known that coronary artery occlusion and reperfusion can lead to cardiogenic shock, bradycardia and arrhythmias, all of which may be fatal complications secondary to acute myocardial infarction. Similar observations were made in the present study in the dog in which coronary artery occlusion and reperfusion soon led to a marked reduction in arterial blood pressure, bradycardia and malignant ventricular arrhythmias. Sinus bradycardia commonly occurs during the early phases of acute myocardial infarction, secondary to bradyarrhythmias or because of extensive damage to the heart with destruction of the conduction pathway. The post-occlusion decreases in arterial blood pressure in control and (+)-naloxone-treated animals were very marked. This may be due to extensive ischaemic injury of the heart, as blood pressure and heart rate (rather high) are within normal ranges (Bolton, 1975) in the pre-occlusion period. Moreover, adequate anaesthesia was maintained during the entire experimental period, using the same dose of pentobarbitone sodium (25 mg kg^{-1}, i.v.) as used in previous studies (Huang *et al.*, 1986; Sakamoto *et al.*, 1989). In addition, the absence of purposeful movement and no tachycardia or pressor responses to leg pinch or pinprick indicated that adequate anaesthesia was maintained throughout the experiment.

In this study, (−)-naloxone prevented the hypotension, bradycardia, ischaemic and reperfusion arrhythmias due to coronary artery occlusion and reperfusion in the dog. These results are in agreement with those obtained in the rat (Fagbemi *et al.*, 1982; Lee *et al.*, 1992) and by Huang *et al.* (1986) in the dog, subjected to myocardial ischaemia and reperfusion. It is of interest to note that the doses of naloxone that produced antiarrhythmic effects in rats and dogs were of a similar order of magnitude. We have previously shown that both β-endorphin (Lee *et al.*, 1984) and dynorphin (Lee & Wong, 1987) are arrhythmogenic in the rat isolated heart, which is the first piece of evidence suggesting that EOP may be involved in cardiac arrhythmogenesis. The second piece of evidence in support of this suggestion is the demonstration of an antiarrhythmic effect of naloxone both *in vivo* (Fagbemi *et al.*, 1982, Lee *et al.*, 1992) and *in vitro* (Zhan *et al.*, 1986; Sarne *et al.*, 1988). However, the possibility that naloxone exerts its antiarrhythmic action via its membrane stabilizing effect rather than by opiate antagonism cannot be excluded. The finding in the present study that (−)-naloxone is antiarrhythmic whilst the isomer lacking opiate antagonistic actions is not, provides more compelling evidence that EOP are indeed involved in the pathophysiology of myocardial ischaemia and reperfusion and that the opiate antagonism is responsible for the antiarrhythmic effect.

The antiarrhythmic effect of naloxone may result from the influence of the central nervous system on the opioid receptors, vagal reflex, prevention of post-occlusion hypotension with a consequent improvement in coronary blood flow, as well as from the receptors located in the heart (Ehrenpresis, 1976; Bergey & Bell, 1983; Liu *et al.*, 1992). Brasch (1986) has demonstrated that naloxone increased both the cardiac action potential duration and the functional refractory period, thus rendering the heart less vulnerable to cardiac arrhythmias. He suggested that naloxone exerted a negative chronotropic effect due to the inhibition of the time-dependent membrane potassium outward current. Others reported prolongation of the conductance in the frog node of Ranvier (Carratu & Mitolo-Chieppa, 1982), and rat heart (Sarne *et al.*, 1988), which suggested inhibition of the inward sodium or calcium currents. In addition, naloxone has a significant influence on the electrophysiological properties of the proximal part of the heart conduction system. It has been reported to lengthen the sinoatrial, intra-atrial and atrioventricular node conduction times, and to prolong the atrial and atrioventricular node effective refractory periods (Markiewicz *et al.*, 1991).

The present results can be explained on the basis that blockade of opioid receptors, perhaps in the myocardium itself, inhibits ischaemia- and reperfusion-induced arrhythmias by reducing the effects of EOP released as a consequence of the stress of myocardial ischaemia and reperfusion. This is in agreement with the recent finding by Parratt & Sitsapesan (1986) who found that two opiate antagonists, (−)-Mr1452 and (−) WIN 44,441-3 are antiarrhythmic while their isomers, without opiate antagonistic properties, are not. They are not compatible, however, with the finding of Sarne *et al.* (1988) that both stereoisomers of naloxone (with and without opiate antagonistic properties) reduced the incidence and severity of cardiac arrhythmias induced in rats by intracarotid administration of adrenaline. This discrepancy may suggest that different mechanisms may be involved in these two events (ischaemia- and adrenaline-induced arrhythmogenesis). Further studies are needed to define the extent of involvement of EOP in cardiac arrhythmogenesis and to elucidate both the mechanisms of action of EOP in the pathophysiology of myocardial ischaemia and reperfusion, and the electrophysiological effects of opioid receptor activation and blockade in cardiac muscle.

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