The effects of drugs interacting with opioid receptors on the early ventricular arrhythmias arising from myocardial ischaemia

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- 1 The effects of a range of opioid receptor agonists and antagonists with differing opioid receptor selectivities on ischaemia-induced arrhythmias in anaesthetised rats was investigated.
- 2 Naloxone was antiarrhythmic only at doses expected to antagonise κ - and δ -receptors in addition to μ -receptors.
- 3 The opioid receptor antagonist Mr 2266, which is twice as potent at κ -receptors as at μ -receptors dose-dependently reduced the incidence and severity of the arrhythmias resulting from coronary artery occlusion.
- 4 The opioid receptor antagonist M 8008 (1 mg kg^{-1}), which is twice as potent at δ -receptors as at μ -receptors but has very little affinity for the κ -receptor, did not exhibit any beneficial antiarrhythmic properties.
- 5 MrZ 2593, a quarternary complex of naloxone which does not readily cross the blood brain barrier, was antiarrhythmic which implies that the antiarrhythmic actions of opioid receptor antagonists may be mediated via peripheral opioid receptors.
- 6 The agonists, diamorphine, [Leu] enkephalin and U-50,488H exhibited no significant arrhythmogenic effects under the present experimental conditions.
- 7 It is tentatively suggested that blockade of peripheral κ -receptors during acute myocardial ischaemia may result in an antiarrhythmic effect.

Introduction

It has previously been shown that naloxone (Fagbemi *et al.*, 1982) and meptazinol (Fagbemi *et al.*, 1983) reduce the incidence and severity of ventricular arrhythmias resulting from acute coronary artery occlusion in both conscious and anaesthetised rats. To explore the possibility that this action was receptor-mediated, the effects of two opioid receptor antagonists ((-)-WIN, 44,441-3 and (-)-Mr 1452) and their stereoisomers ((+)-WIN, 44,441-2 and (+)-Mr 1453) on ischaemia-induced arrhythmias in anaesthetised rats were investigated (Parratt & Sitsapesan, 1986). (-)-Mr 1452 but not (+)-Mr 1453 reduced, in a dose-dependent manner, the number of ventricular ectopic beats and the incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF). A similar protective effect was observed with (-)-WIN 44,441,3 but not with (+)-WIN 44,441-2. Thus, these results indicated that a specific receptor-

mediated effect was responsible for the beneficial effects of opioid receptor antagonists under conditions of myocardial ischaemia.

If specific opioid receptors are indeed involved to which opioid receptor subtypes do they belong? The present study was designed to attempt to answer this question by investigating the antiarrhythmic properties of a range of opioid antagonists with varying opioid receptor selectivities. The ligands used for this purpose were naloxone (most potent at μ -receptors, Paterson *et al.*, 1983), Mr 2266 (mainly μ and κ , Paterson *et al.*, 1983; Smith *et al.*, 1984) and M 8008 (μ and δ , Smith, 1987). The effects of Mr Z 2593, a quarternary complex of naloxone which does not readily enter the central nervous system (Tavani *et al.*, 1979; Bianchi *et al.*, 1982), were also investigated in an attempt to assess the importance of peripherally located opioid receptors.

Table 1 The effects of naloxone on the severity of the ventricular arrhythmias that occurred over the 0–30 min post-occlusion period

Group	n	Ventricular ectopic count	Duration of VT (s)	Duration of VF (s)	% VT	% VF	% mortality
Controls	11	583 ± 238	27.2 ± 12	5.4 ± 3.7	82	36	0
Naloxone 50 µg kg ⁻¹ + 0.25 µg kg ⁻¹ min ⁻¹	9	582 ± 163	29.6 ± 7.7	9.4 ± 9.1	89	44	11
Controls	7	1087 ± 305	64.9 ± 26	8.9 ± 4.7	100	57	0
Naloxone 0.5 mg kg ⁻¹ + 0.25 µg kg ⁻¹ min ⁻¹	11	*213 ± 70	*4.5 ± 1.4	5.8 ± 3.2	64	27	0
Controls	10	1307 ± 346	60.4 ± 23	21.8 ± 10.9	100	90	20
Naloxone 2.0 mg kg ⁻¹ + 1 µg kg ⁻¹ min ⁻¹	10	*463 ± 144	15.7 ± 5.6	7.7 ± 6.1	60	*30	0
Controls	15	1305 ± 320	70.6 ± 21.9	49.1 ± 20	100	67	13
Naloxone 5.0 mg kg ⁻¹ + 2.5 µg kg ⁻¹ min ⁻¹	13	591 ± 152	26.8 ± 9.2	6.0 ± 3.1	92	42	0
Controls	9	1258 ± 428	70.6 ± 21.9	0	100	0	0
MrZ 2593 1 mg kg ⁻¹	10	*270 ± 69	*10.8 ± 4.6	0	100	0	0

The mean values ± s.e.mean for the ventricular ectopic count, the incidence and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) and mortality are also shown. Values for the ventricular ectopic count and the duration of VT and VF are taken only from rats that survived beyond 30 min. *n* = number of animals.

* *P* < 0.05.

If opioid receptor antagonists are antiarrhythmic as a result of their antagonistic effects on endogenously released opioid peptides, it would seem appropriate to investigate the possibility that opioid agonists may be arrhythmogenic under similar conditions of ischaemia. This was examined using diamorphine (µ-agonist, Smith, 1984) U-50,488H (κ-agonist, Von Voightlander *et al.*, 1983) and [Leu] enkephalin which has δ-agonist properties (Paterson *et al.*, 1983). Preliminary accounts of some of these findings has been given to a meeting of the British Pharmacology Society (Mackenzie *et al.*, 1986a,b).

Methods

The method used was basically that described by Clark *et al.* (1980). Male Sprague-Dawley rats weighing between 250 and 350 g were anaesthetised with pentobarbitone sodium (6 mg 100 g⁻¹ i.p.) and artificially ventilated with room air (54 strokes min⁻¹; stroke volume 2 ml 100 g⁻¹). Catheters were placed in a carotid artery (for pressure measurement) and in a femoral vein (for drug injection) and the electrocardiogram was recorded from standard limb leads. Rectal temperature was maintained at approximately 38°C. The chest was opened between the fourth and fifth ribs, approximately 2 mm to the left of the sternum. After opening the pericardium the heart was exteriorized and a 6/0 silk suture was placed under the left coronary artery. The heart was repositioned in the thoracic cavity and a 15 min stabilisation period was allowed. Drugs or vehicle were

administered 15 min before coronary artery occlusion. The electrocardiogram and blood pressure were recorded for 30 min after occlusion of the coronary artery. The severity of the arrhythmias was assessed by counting the total number of ventricular extrasystoles occurring in the 30 min post-occlusion period and by measuring the duration and incidence of ventricular fibrillation (VF), ventricular tachycardia (VT) and mortality.

Statistics

Statistical analysis of differences in the incidence of arrhythmias and in mortality was carried out by use of the Fisher Exact Probability Test. For analysis of the difference between means Student's *t* test was used when one control group was compared with one test group, when more than one test group was compared with a single control group, analysis of variance plus Bartlett's test, followed by Dunnett's test was used. For non-parametric data, the Kruskal-Wallis test followed by the Mann-Witney-U test was used.

Drugs

The following drugs were used: naloxone hydrochloride (Salas, Italy), Mr 2266 (5,9-diethyl-2-(3-oxazolylmethyl)benzomorphan), (Dr H. Merz, Boehringer Ingelheim), M 8008 (N-cyclopropylmethyl-16(R)-methylmethylorvinol), (Reckitt & Colman, Hull), MrZ 2593 (1-N-allyl-N-methyl-7,8-dihydro-14-hydroxynormorphinone bromide), (Dr H. Merz, Boehringer, Ingelheim) diamorphine hydro-

chloride (Reckitt & Colman, Hull), [Leu]enkephalin (Cambridge Research Biochemicals), U-50,488H (N-methyl - N' - (pyrrolidinyloxy) - 3,4 - dichloro - benzylamide), (Upjohn).

Results

The effects of opioid receptor antagonists on the consequences of coronary artery occlusion

Table 1 shows the effects of naloxone on the severity of ventricular arrhythmias, and on mortality, resulting from coronary artery occlusion. The lowest dose of naloxone used ($50 \mu\text{g kg}^{-1}$ given 15 min before ligation together with an infusion of $0.25 \mu\text{g kg}^{-1} \text{min}^{-1}$ commencing at this time and continuing throughout the occlusion period) had no significant effect on ventricular arrhythmias. Doses of 2 mg kg^{-1} then $1 \mu\text{g kg}^{-1} \text{min}^{-1}$ and 0.5 mg kg^{-1} then $0.25 \mu\text{g kg}^{-1} \text{min}^{-1}$ naloxone reduced the number of ventricular extrasystoles from 1307 ± 346 to 463 ± 144 ($P < 0.05$) and from 1078 ± 305 to 213 ± 70 ($P < 0.05$) respectively. Naloxone (0.5 mg kg^{-1} then $0.25 \mu\text{g kg}^{-1} \text{min}^{-1}$) also reduced the duration of VT from 65 ± 26 to 4.5 ± 1.4 s ($P < 0.05$) and, in a higher dose (2 mg kg^{-1} then $1 \mu\text{g kg}^{-1} \text{min}^{-1}$) reduced the incidence of VF from 90% to 30%. The quaternary derivative of naloxone, Mr Z 2593, at a dose of 1 mg kg^{-1} , was also antiarrhythmic (Table 1). It reduced significantly the ventricular ectopic count from 1258 ± 428 to 270 ± 69 ($P < 0.05$) and the duration of VT from 117.9 ± 42.8 to 10.8 ± 4.6 s. Neither naloxone nor Mr Z 2593 significantly modified heart rate or mean arterial blood pressure (MABP) before or after coronary artery occlusion. For example, in control animals blood pressure fell 1 min after occlusion from 106 ± 6 mmHg to 79 ± 4 mmHg, with recovery to 103 ± 5 mmHg at 15 min whereas in those rats pretreated with naloxone (2 mg kg^{-1} then $1 \mu\text{g kg}^{-1} \text{min}^{-1}$) blood pressure fell on occlusion from 89 ± 5 mmHg to 68 ± 4 mmHg at 1 min with recovery to 82 ± 7 mmHg at 15 min.

Like naloxone, Mr 2266 also caused a dose-dependent reduction in the number of ventricular extrasystoles and in the duration of VT (Figure 1). For example, Mr 2266 at a dose of 4 mg kg^{-1} reduced the number of ventricular ectopic beats from 1111 ± 238 to 246 ± 74 ($P < 0.01$) and the duration of ventricular tachycardia from 69 ± 23 to 7 ± 3.5 s. Mr 2266 had little effect on arterial pressure but did transiently reduce heart rate (464 ± 23 beats min^{-1} to 437 ± 20 beats min^{-1} with a dose of 2 mg kg^{-1} and 435 ± 20 beats min^{-1} to 331 ± 12 beats min^{-1} with a 4 mg kg^{-1} dose).

M8008 (1 mg kg^{-1}) did not modify the total number of ventricular extrasystoles (754 ± 206

Figure 1 The mean ventricular ectopic count (b) and duration of ventricular tachycardia (a) in control and Mr 2266-treated rats. The mean values are shown and vertical lines indicate s.e.mean. ** $P < 0.01$. Open columns, controls; hatched columns, Mr 2266-treated rats.

($n = 11$) in the controls; 608 ± 129 ($n = 10$) in the treated group), the duration and incidence of either VT (59 ± 19 vs 46 ± 12 s (NS)) or VF (8.4 ± 8.0 vs 0 s (NS)), blood pressure (79 ± 5 mmHg before administration and 85 ± 4 mmHg after) and heart rate (from 376 ± 17 beats min^{-1} to 370 ± 16 beats min^{-1}).

The effects of opioid receptor agonists on the consequences of coronary artery occlusion

In the doses used, diamorphine, U-50,488H and [Leu] enkephalin had no significant effect on the early ventricular arrhythmias resulting from coronary artery occlusion (Table 2).

Table 2 The effects of diamorphine, [Leu] enkephalin and U-50,488H on the severity of the ventricular arrhythmias that occurred over the 0–30 min post-ligation period

Group	n	Ventricular ectopic count	Duration of VT (s)	Duration of VF (s)	% VT	% VF	% mortality
Controls	10	1088 ± 328	65.6 ± 26.0	4.7 ± 2.7	100	40	0
Diamorphine (0.05 mg kg ⁻¹)	10	983 ± 474	54.5 ± 27.0	1.7 ± 1.3	100	50	20
Diamorphine (0.20 mg kg ⁻¹)	10	814 ± 276	50.1 ± 21.8	7.0 ± 5.7	90	20	0
Diamorphine (0.50 mg kg ⁻¹)	10	622 ± 163	32.2 ± 11.6	8.1 ± 8.1	100	30	20
Controls	10	1325 ± 347	86.9 ± 27.0	22.6 ± 12.9	100	50	10
[Leu] enkephalin							
0.1 µg kg ⁻¹ min ⁻¹	10	644 ± 109	33.8 ± 9.4	15.8 ± 8.1	100	50	0
0.5 µg kg ⁻¹ min ⁻¹	9	760 ± 234	36.9 ± 14.9	18.9 ± 14.5	100	56	11
2 µg kg ⁻¹ min ⁻¹	9	1430 ± 349	83.2 ± 25.3	37.6 ± 16.9	100	67	0
10 µg kg ⁻¹ min ⁻¹	8	1187 ± 371	63.9 ± 24.1	29.3 ± 11.2	100	75	0
Controls	10	1284 ± 385	88.9 ± 34.1	20.9 ± 8.3	100	70	10
U-50,488H (1 mg kg ⁻¹)	10	924 ± 342	54.4 ± 23.9	7.6 ± 3.9	100	30	0
<i>Infusions</i>							
Controls	10	994 ± 331	57.0 ± 22.4	16.6 ± 8.3	80	50	0
U-50,488H (0.1 mg kg ⁻¹)	10	881 ± 201	53.4 ± 15.4	12.4 ± 6.9	100	50	0
U-50,488H (0.3 mg kg ⁻¹)	9	779 ± 281	40.0 ± 17.6	5.8 ± 4.2	100	22	0

Mean values ± s.e.mean are shown. *n* = number of animals.

Table 3 summarises the effects of diamorphine and U-50,488H on blood pressure and heart rate. [Leu] enkephalin was without significant effects on these parameters in doses up to 2 µg kg⁻¹ min⁻¹; at the highest dose used (10 µg kg⁻¹ min⁻¹) there was a transient, slight increase in arterial pressure (from 100 ± 4 to 109 ± 8 mmHg; *P* < 0.05). The administration of diamorphine resulted in gradual and sustained increases in both blood pressure and heart rate such that just before coronary artery occlusion these were significantly higher than those in the controls. Diamorphine also attenuated the decrease in pressure which normally resulted from occlusion (Table 3). U-50,488H (1 mg kg⁻¹) given as a bolus dose 15 min before coronary artery occlusion markedly decreased arterial blood pressure and heart rate. Smaller doses given by infusion also tended to decrease rate.

Discussion

In a previous study by Parratt & Sitsapesan (1986), WIN,44,441-3 (an antagonist with a similar profile to naloxone, Ward *et al.*, 1983) and Mr 1452 (a relatively non-selective opioid antagonist, Smith *et al.*, 1984) were shown to be antiarrhythmic in anaesthetised rats. In the present study, antagonists which act preferentially at μ - or κ -receptors (i.e. naloxone, Mr 2266) were antiarrhythmic whereas M 8008 which is twice as potent at δ -receptors than at μ -receptors and which has little affinity for κ -receptors

(Smith, 1987) was without effect. These results suggest that δ -receptors are unlikely to be involved in the antiarrhythmic actions of opioid antagonists. At least ten times more naloxone is required to antagonise κ - or δ -receptors than is required to antagonise μ -receptors (Lord *et al.*, 1977; Robson *et al.*, 1983). Work by other investigators (e.g. Leander, 1983) would suggest that, at the doses of naloxone which were antiarrhythmic in this model, naloxone was antagonising δ - and κ -receptors in addition to μ -receptors.

When a lower dose (50 µg kg⁻¹ + 0.25 µg kg⁻¹ min⁻¹), calculated to antagonise μ -receptors only, was used, naloxone was not antiarrhythmic. On the basis of these results it is suggested that κ -receptors may be important in mediating the antiarrhythmic actions of opioid receptor antagonists. A peripheral site of action of these antagonists is suggested, but certainly not proved, by the observation that Mr Z 2593, a quarternary naloxone derivative that does not easily cross the blood brain barrier, was also antiarrhythmic. Furthermore, Zhan *et al.* (1985) have demonstrated an antiarrhythmic action of naloxone in the rat isolated heart.

The characterisation of the opioid receptor subtype(s) mediating the antiarrhythmic effect of these antagonists has important clinical implications. An opioid analgesic acting via stimulation of μ -receptors is usually administered during acute myocardial infarction. Thus, on the basis of these experimental results it may be of more benefit to administer a drug possessing both μ -receptor agonist

Table 3 The effect of diamorphine and U-50,488H on heart rate and mean arterial blood pressure before and at various times after coronary artery ligation in anaesthetised rats

Group	n	Heart rate (beats min ⁻¹)				
		Time post-ligation (min)				
		-15	-10	0	1	15
Controls	10	398 ± 24	375 ± 22	395 ± 13	414 ± 22	414 ± 15
Diamorphine (0.05 mg kg ⁻¹)	10	375 ± 19	430 ± 16	450 ± 9*	428 ± 15	441 ± 9
Diamorphine (0.2 mg kg ⁻¹)	10	400 ± 12	447 ± 12*	468 ± 6*	463 ± 7	455 ± 10
Diamorphine (0.5 mg kg ⁻¹)	10	402 ± 16	413 ± 15	436 ± 11	442 ± 13	420 ± 16
Controls	10	461 ± 15	446 ± 17	461 ± 12	462 ± 12	460 ± 12
U-50,488H (0.1 mg kg ⁻¹ min ⁻¹)	10	450 ± 15	451 ± 14	437 ± 14	435 ± 16*	443 ± 12
U-50,488H (0.3 mg kg ⁻¹ min ⁻¹)	9	436 ± 12	440 ± 13	426 ± 11*	414 ± 15*	406 ± 13**
Controls	10	361 ± 7	377 ± 18	374 ± 14	366 ± 11	408 ± 17
U-50,488H (1 mg kg ⁻¹)	10	348 ± 14	308 ± 18**	314 ± 13**	301 ± 11*	323 ± 12**

Group	n	Mean arterial blood pressure (mmHg)				
		Time post-ligation (min)				
		-15	-10	0	1	15
Controls	10	94 ± 6	98 ± 6	101 ± 4	81 ± 7	110 ± 8
Diamorphine (0.05 mg kg ⁻¹)	10	88 ± 6	129 ± 8*	133 ± 4*	97 ± 4	117 ± 6
Diamorphine (0.2 mg kg ⁻¹)	10	74 ± 3	137 ± 4*	143 ± 4*	131 ± 5	131 ± 5
Diamorphine (0.5 mg kg ⁻¹)	10	86 ± 3	116 ± 7	120 ± 7	116 ± 8*	118 ± 10
Controls	10	90 ± 5	96 ± 6	113 ± 7	86 ± 4	88 ± 2
U-50,488H (0.1 mg kg ⁻¹ min ⁻¹)	10	90 ± 3	107 ± 4	104 ± 5	80 ± 3	91 ± 7
U-50,488H (0.3 mg kg ⁻¹ min ⁻¹)	9	100 ± 4	114 ± 6	110 ± 5	77 ± 3	91 ± 6
Controls	10	77 ± 1	95 ± 6	83 ± 6	69 ± 3	83 ± 8
U-50,488H (1 mg kg ⁻¹)	10	77 ± 2	64 ± 4**	70 ± 3*	47 ± 2**	53 ± 4**

The mean values ± s.e.mean are shown, *n* = number of animals. **P* < 0.05; ***P* < 0.01.

and κ -receptor antagonist properties. Indeed, it has been shown that analgesics such as meptazinol and buprenorphine, which are partial agonists at μ -receptors also possess antiarrhythmic actions (Fagbemi *et al.*, 1983; Sitsapasan *et al.*, 1987).

The agonists used in this study, U-50,488H (κ -agonist, Von Voightlander *et al.*, 1983), [Leu] enkephalin (most potent at δ -receptors, Paterson *et al.*, 1983) and diamorphine (μ -agonist, Smith, 1984) were without effect on the arrhythmias resulting from coronary artery occlusion. One possible reason for this lack of effect may be that the opioid receptors concerned are maximally stimulated by endogenous opioids released during the period of ischaemia. It is of interest to note that in rat isolated perfused hearts, in which opioid release could be small, β -endorphin has been shown to cause arrhythmias (Lee *et al.*, 1984). Another possibility is that the reduction in heart rate observed with the κ -agonist,

U-50,488H, or the ability of diamorphine to attenuate the fall in arterial blood pressure that occurs upon occlusion may have obscured any direct arrhythmogenic effect. It could also be argued that the antiarrhythmic action of these opioid receptor antagonists is not mediated via opioid receptors but is due to a direct electrophysiological effect. Recent work by Brasch (1986) has demonstrated that both (+) and (-)-isomers of naloxone may prolong the action potential duration of guinea-pig papillary muscle, an effect which is considered to be antiarrhythmic. On the other hand, this explanation does not take account of the observed stereospecificity of the antiarrhythmic action of the opioid receptor antagonists (Parratt & Sitsapasan, 1986). Further work is obviously required in this area to assess the relative importance of opioid receptor mediated and direct membranous effects of these drugs underlying their antiarrhythmic properties.

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