Anti-arrhythmic activities of opioid agonists and antagonists and their stereoisomers

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- 1 A series of opioid agonists, antagonists and their (+)-stereoisomers were tested for antiarrhythmic activity in the rat coronary artery occlusion model.
- 2 Naloxone (0.01–2 mg kg⁻¹) significantly reduced the incidence and severity of cardiac arrhythmias, in accordance with previous published studies.
- 3 The non-opioid stereoisomer, (+)-naloxone, was equipotent with naloxone against occlusion-induced arrhythmia.
- 4 Similar non-stereospecific antiarrhythmic effects were induced by another opioid antagonist, Win 44,441-3 and its stereoisomer Win 44,441-2.
- 5 The opioid agonists, morphine and levorphanol, protected against occlusion-induced arrhythmia as did the opioid antagonists, and the (+)-stereoisomer, dextrorphan, was equipotent to levorphanol.
- 6 It is concluded that the antiarrhythmic effects of opioid drugs are not mediated by opioid receptors. A direct effect on ionic currents in cardiac muscle is suggested as the mechanism of opioid antiarrhythmic activity.

Introduction

The opioid antagonist, naloxone, has been shown previously to reduce the incidence and severity of cardiac arrhythmias resulting from coronary ligation in rats (Fagbemi *et al.*, 1982). Similar antiarrhythmic activity of naloxone was later described in other species with various experimental models (Huang *et al.*, 1986; Lee *et al.*, 1986). The accepted explanation for this action of naloxone is the blockade of opioid receptors which prevents the putative disturbing effects of endogenous opioid peptides. We recently found, however, that the non-opioid stereoisomer (+)-naloxone is as effective as (–)-naloxone in abolishing arrhythmias, indicating that the antiarrhythmic activity of the drug is not mediated by opioid receptors (Sarne *et al.*, 1988). Our conclusions contradicted the findings of Parratt & Sitsapasan (1986), who showed that two other opioid antagonists, Win 44,441 and Mr 1452, protect against cardiac arrhythmia in a stereospecific manner. The stereospecificity, however, was much lower than expected for opioid receptors (see Discussion for details).

A major difference between these two aforementioned studies is the model of arrhythmia: we studied adrenaline-induced arrhythmia while Parratt & Sitsapasan (1986) used coronary artery occlusion. In order to compare the two studies, and to elucidate the mechanism by which opiates exert their antiarrhythmic activity, we tested the effects of various opioid agonists and antagonists in a model of coronary occlusion-induced arrhythmia. The results support our hypothesis that the antiarrhythmic action of opiates does not involve opioid receptors.

Methods

Charles River descendent male rats (400–500 g) were anaesthetized intraperitoneally with pentobarbitone (30 mg kg⁻¹). The femoral vein was cannulated for administration of drugs, and the electrocardiogram (lead II) was recorded by limb electrodes. Coronary artery occlusion was carried out as described by Clark and co-workers (1980).

Drugs were administered intravenously in 0.1–1.0 ml of dextrose, followed, 2 min later, by the ligation of the cardiac

artery. The electrocardiogram was monitored for 90 min, or until death of the animal.

The dysrhythmias identified in the electrocardiogram included ventricular ectopic beats (VEBs), ventricular tachycardia (VT) and ventricular fibrillation (VF). Arrhythmia was defined as more than 10 VEBs or a dysrhythmic incidence (VT or VF) of more than 10 s duration.

The following drugs were used: the opioid antagonists naloxone and Win 44,441-3 (guadazocine) and their stereoisomers (+)-naloxone and Win 44,441-2; the opioid agonists morphine and levorphanol and the (+)-stereoisomer dextrorphan. Each experiment consisted of 6 groups of 5–8 animals each: the control group received dextrose, and the other 5 groups received various drugs in different doses. The experiments were carried out double blind and drugs code was revealed only after the electrocardiogram had been analyzed.

The Chi Square and Fisher Exact Probability tests were used for comparing proportions, and the Mann-Whitney test provided non-parametric quantitative comparisons (Siegel, 1956).

Results

Coronary artery occlusion elicited severe arrhythmias in 38 of 51 control rats (74%); 17 of these rats (44%) died before the end of the experiment (90 min). The number of VEBs in control animals during the first 10 min following occlusion was 41.1 ± 37 (Table 1).

Administration of 2 mg kg⁻¹ naloxone 2 min before ligation reduced the incidence and severity of cardiac arrhythmia: only 7 out of 22 (32%) showed severe arrhythmia, with 4 rats (18%) dying in 90 min. The number of VEBs in the naloxone-treated group was 2.5 ± 5 . All the examined parameters were significantly lower in the naloxone-treated group than in control animals. A lower dose of naloxone, 0.01 mg kg⁻¹, was still effective in protecting rats from arrhythmia, while 0.001 mg kg⁻¹ naloxone offered no protection (Table 1). The non-opiate isomer (+)-naloxone showed similar protective effects and dose-dependency (Table 1).

Moderate doses (0.1 mg kg⁻¹) of the opioid antagonist Win 44,441-3 and its stereoisomer Win 44,441-2 were similarly effective in preventing arrhythmias (Table 1). Higher doses of these drugs (1–4 mg kg⁻¹) were not effective and sometimes induced arrhythmias and caused death even without ligation

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Table 1 The effect of opioid agonists, antagonists and stereoisomers on coronary occlusion-induced arrhythmias

Drug	Dose (mg kg ⁻¹)	n	Arrhythmic rats (%)	VEBs in 10 min
Control	—	51	74	41.1
Naloxone	2	22	32**	2.5**
	0.01	8	37*	5.9*
	0.001	5	60	78.8
(+)–Naloxone	2	7	14**	4.0**
	0.01	6	16**	4.5*
Win 44,441-3	2	9	100	45.9
	0.1	11	27*	3.2**
	0.01	10	50	53.4
Win 44,441-2	0.1	7	14**	4.3**
	0.01	5	40	51.0
	0.2	6	66	79.5
Levorphanol	0.05	5	20*	5.4*
	0.005	5	20*	0.4**
	0.05	5	40	15.0
Dextrorphan	0.005	5	20*	0.8**
	4	7	29*	6.7
Morphine	0.2	12	83	19.5

VEBs: ventricular ectopic beats.

Significantly less than control: * $P < 0.05$, ** $P < 0.01$.

of the coronary artery. These arrhythmogenic effects of Win 44,441 were associated with a fall in blood pressure and led to death in a few animals, similar to the lethal side-effects of high doses of other known antiarrhythmic drugs (Bigger & Hoffman, 1990).

The opioid agonist, levorphanol, was also an effective antiarrhythmic agent: 0.005 mg kg⁻¹ reduced the incidence of severe arrhythmias to 20%, compared to 74% in controls, and the number of VEBs to 0.4, compared to 41.1 in controls. Higher doses (0.05 mg kg⁻¹) were somewhat less effective, while a dose of 0.2 mg kg⁻¹ elicited arrhythmias even without coronary occlusion. Similar results were obtained with the non-opioid stereoisomer dextrorphan.

The classical opioid agonist, morphine, exhibited similar antiarrhythmic activity, although a significant effect could be obtained only at relatively high doses (2–4 mg kg⁻¹).

Discussion

The present study confirms the original findings of Fagbemi *et al.* (1982) on the antiarrhythmic activity of naloxone. The pharmacological effects of naloxone are commonly attributed to the blockade of opioid receptors which prevents the action of endogenous opioid peptides, an explanation supported by Parratt & Sitsapesan (1986) who showed that the (+)-isomers Win 44,441-2 and Mr 1453 were less effective than their (–)-opioid antagonist counterparts. Quantitative examination of their results, however, reveals that Mr 1453 was only 2–10 times less potent than its opioid stereoisomer Mr 1452. The expected ratio of potency between these two isomers at the opioid receptor is 300 (Merz *et al.*, 1974).

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- An alternative explanation for the antiarrhythmic action of naloxone is a direct, non-opioid effect on the cardiac muscle. This hypothesis was supported by our finding (Sarne *et al.*, 1988) that (+)-naloxone, which is 1000–10,000 less potent as an opioid antagonist (Iijima *et al.*, 1978) is equipotent as an antiarrhythmic agent. We decided to verify the results of our previous study which was carried out on adrenaline-induced arrhythmia, by testing it with the more conventional, coronary occlusion model used by Parratt & Sitsapesan. The present study confirms our earlier findings and supports our hypothesis that the antiarrhythmic effect of naloxone does not involve opioid receptors. Furthermore, similar results were obtained in the present study with another pair of opioid antagonist isomers, Win 44,441-3 and Win 44,441-2.
- This hypothesis gains additional support from our finding that opioid agonists are also effective antiarrhythmic agents. Both morphine and levorphanol protected the animals from ligation-induced arrhythmia, similar to the opioid antagonists. This effect was also not stereospecific with the non-opioid (+)-isomer, dextrorphan, equipotent to levorphanol.
- While our results seem to contradict previous findings by Parratt & Sitsapesan (1986), it must be remembered that the two studies differ in several ways, including strains of rats, timing of drug administration and severity of the induced arrhythmias. In our present model, as well as in our previous studies on adrenaline-induced arrhythmia (Sarne *et al.*, 1988) and ouabain-induced arrhythmia (Sarne *et al.*, unpublished observations), the antiarrhythmic activity of opiates was not stereospecific. Parratt & Sitsapesan (1986), on the other hand, observed some stereospecificity, albeit much lower than expected for opioid receptors. It is feasible that two different mechanisms mediate the antiarrhythmic activity of opiates, one that involves opioid receptors and another non-opioid in nature. The latter mechanism predominates in our model, while both mechanisms are involved in Parratt & Sitsapesan's model, leading to partial opioid stereospecificity.
- The mechanism by which opioid agonists and antagonists exert their non-opioid antiarrhythmic activity can be explained by our *in vitro* studies on cardiac muscle (Sarne, 1989) in which opiates were found to suppress excitability of isolated strips of muscles in a non-stereospecific fashion. These findings are in accord with previous studies on the suppression of sodium and potassium currents by opioid drugs (Frazier *et al.*, 1973; Carratu & Mitolo-Chieppa, 1982; Brasch, 1986). The molecular basis for this effect is currently under investigation in our laboratory. Preliminary results with radiolabelled markers indicate that opioid agonists as well as opioid antagonists interact with voltage-dependent sodium channels, and that this interaction is not mediated by opioid receptors. This non-opioid action of opiates should be taken into consideration when interpreting the pharmacological effects of opioid drugs.

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