J Mol Cell Cardiol 22, 1167–1175 (1990)

Effects of Drugs Interacting with Opioid Receptors During Normal Perfusion or Ischemia and Reperfusion in the Isolated Rat Heart—an Attempt to Identify Cardiac Opioid Receptor Subtype(s) Involved in Arrhythmogenesis

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(Received 18 October 1989, accepted in revised form 10 May 1990)

T. M. Wong, A. Y. S. Lee and K. K. Tai Effects of Drugs Interacting with Opioid Receptors During Normal Perfusion or Ischemia and Reperfusion in the Isolated Rat Heart—an Attempt to Identify Cardiac Opioid Receptor Subtype(s) Involved in Arrhythmogenesis. *Journal of Molecular and Cellular Cardiology* (1990) **22**, 1167–1175. Cardiac opioid receptors have been shown to be involved in the genesis of arrhythmias during ischemia and reperfusion. The present study was aimed at elucidating the receptor subtype(s) involved in arrhythmogenesis. Two series of experiments were conducted. In the first, effects of prototype opioid agonists, namely, (D-Ala^2, NMe^4, Gly-ol)-Enkephalin (DAGO), U50,488H and (D-Pen^2, Pen^5)-Enkephalin (DPDPE) and (D-Ala^2, D-Leu^2)-Enkephalin (DADLE), representing μ-, k- and δ-agonists, respectively, in disturbing the normal cardiac rhythm in the isolated perfused rat heart were investigated. Both DAGO and U50,488H were arrhythmogenic, whereas the effects of the δ-agonists, DPDPE and DADLE at a same dose range (44–396 nmol/heart) as that of DAGO were almost negligible. U50,488H was by far the most potent as it induced ventricular arrhythmias including frequent PVC and VT even at a dose (44 nmol/heart) at which other agonists either produced no or negligible effect. In the second series of experiments, the antiarrhythmogenic effects of μ-antagonist (naloxone) and k-antagonist (MR 2266) against arrhythmias arising during ischemia and reperfusion were compared. The effects of MR 2266 were significantly greater than that of naloxone. Results of the present study suggest that the cardiac k-receptors are the most likely receptor-subtype involved in arrhythmogenesis during ischemia and reperfusion.

Key Words: Receptor subtype; Opioid agonist; Opioid antagonist; Ischemia; Reperfusion; Isolated rat heart; Arrhythmia.

Introduction

There are several pieces of evidence suggesting that cardiac opioid receptors are activated during ischemia and reperfusion. Firstly, opioid antagonist, naloxone (Zhan *et al.*, 1985; Lee and Wong, 1986; 1987a) or naltrexone (Liu *et al.*, 1988) has been shown to attenuate arrhythmias during ischemia and reperfusion or during reperfusion only in the isolated rat heart. Secondly, isolated perfused hearts of chronically morphine-treated rats exhibit tolerance to dynorphin$_{1-13}$ or ischemia and reperfusion in induction of arrhythmias (Wong and Lee, 1987). Thirdly, buprenorphine, a partial μ-receptor agonist and partial k-receptor antagonist, which is antiarrhythmic during myocardial ischemia in anesthetised rats, affects the generation of action potential in the isolated perfused papillary muscle (Boachie- Ansah *et al.*, 1989), indicating that the blocking action occurs at the myocardial level. The receptor subtype(s) in the heart that is(are) involved is not known although in anesthetised rats, MR 2266, a selective k-antagonist, is found to be the most potent antiarrhythmic agent among the three types of opioid antagonists (Boachie-Ansah *et al.*, 1989), which unfortunately does not provide information on whether or not cardiac opioid receptors are involved. A useful approach to identify the cardiac opioid receptor subtype(s) involved in arrhythmogenesis is

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0022-2828/90/101167+09 $03.00/0

firstly to compare the effects of different types of specific opioid agonists in disturbing the cardiac rhythm and secondly to study the effects of specific opioid antagonists against arrhythmias arising during ischemia and reperfusion in the isolated rat heart preparation.

In the present study, we firstly compared in the isolated rat heart preparation the arrhythmogenic potencies of (D-Ala^2, NMe^4, Gly-ol)-Enkephalin (DAGO), U50,488H, and (D-Pen^2, Pen^5)-Enkephalin (DPDPE) and (D-Ala^2, D-leu^5)-Enkephalin (DADLE), representing μ-, k- and δ-agonists, respectively. Based on the finding that U50,488H a selective k-receptor agonist, and DAGO, a selective μ-agonist, were arrhythmogenic, whereas DADLE and DPDPE at the same dose range as that of DAGO, were almost non arrhythmogenic, we subsequently compared the antiarrhythmic potencies of MR 2266, a selective k-receptor antagonist and naloxone, a selective u-receptor antagonist against arrhythmias during ischemia and reperfusion. The manuscript reported the results of the study. Although all agonist reduced the sinus rate significantly, the effect was not reported as previous studies suggest that a reduction in the sinus rate of the isolated heart to a similar extent does not result in any significant alteration in electrocardiagrams (Huang and Wong, 1989; Wong *et al.*, unpublished data).

Materials and Methods

Animal preparation

Female Sprague–Dawley rats of 190–210 g were used. The heart was removed and mounted to the Langendorff isolated heart apparatus immediately after sacrifice. It was perfused retrogradely with Krebs–Ringer solution at a flow rate of 5–7 ml/min. The temperature was kept at 31–32°C by a water jacket surrounding it. The pH was maintained at 7.4 by a gas mixture of 95% of O_2 and 5% of CO_2. Addition of drugs used in the present study did not alter the pH. The heart was allowed to stabilise for 10 min before experiment. Any heart exhibiting arrhythmia during this period was discarded as the occurrence of arrhythmias suggested that the heart might have been subjected to excessive ischemia during handling. Hearts in the control group did not exhibit any arrhythmia up to 80 min, indicating that 10 min was sufficient for stabilization. In the experiment studying the effects of agonists on cardiac rhythm, observation was performed for 80 min as preliminary experiments in our laboratory showed that most arrhythmias appeared as early as within 1 min to as late as 80 min after drug administration and that after 80 min the incidence of arrhythmias, if they ever occurred, was negligible compared with that occurred before 80 min. Ischemia was produced by stoppage of perfusion for 20 min followed by reperfusion. Twenty minutes was chosen as previous studies in our laboratory showed that myocardial ischemia in the isolated heart for such duration produced sufficient incidence of arrhythmias for the study of antiarrhythmic effects of opioid antagonists without unduely affecting the cardiac functions (Zhan *et al.*, 1985). The effects of antagonists during reperfusion was observed for 60 min as it was shown that the effects of naloxone (Zhan *et al.*, 1985; Lee and Wong, 1987b) and MR 2266 (Wong *et al.*, preliminary observation) occurred well before 60 min after reperfusion.

Measurement and recognition of electrocardiogram (ECG)

ECG was monitored with standard lead II throughout the experiment with a positive and negative electrodes hooked to the apex and the aorta, respectively. In the present study, both atrial arrhythmias including premature atrial contraction (PAC) and atrial-ventricular block (A-V block) and ventricular arrhythmias including premature ventricular contraction (PVC), ventricular tachycardia (VT) and ventricular fibrillation (VF) were observed. VT was defined as a successive run of at least 6 PVCs of uniform QRS complex. If there were irregular waves of varying amplitude and shape, it was considered to be VF. When there were 3 or more PVCs occurring within 1 min, it was considered to be frequent. When less than 3 PVCs occurred in a min, it was occasional.

Arrhythmia scoring system

To enable quantitative comparison a scoring system modified from that of Curtis and

Walker (1988) was employed. The principles of the scoring system adopted were (1) ventricular arrhythmias were more severe than atrial arrhythmias; (2) the severity of ventricular arrhythmias were VF, VT, frequent PVC and occasional PVC in descending order; (3) the longer the duration of arrhythmias or the more frequent the incidence of arrhythmias the greater the severity of arrhythmias. In the present study, the score of a heart was that of the most severe type of arrhythmia the heart exhibited. In the experiment involving ischemia- and reperfusion-arrhythmias, each heart was given one score, representing the most severe type of arrhythmia observed during the entire period. The details of the scoring system are in Table 1.

Agonists and antagonists used

Four prototype opioid agonists were used in the study. They were (D-Ala^2, NMe^4, Gly-ol)-Enkephalin (DAGO, Peninsula Laboratory), a selective u-agonist, U50,488H (Upjohn Co.), a selective k-agonist, and (D-Pen^2, Pen^5)-Enkephalin (DPDPE, Peninsula Laboratory) and (D-Ala^2, D-Leu^5)-Enkephalin (DADLE, Peninsula Laboratory), selective δ-agonists. The opioid antagonists used were MR 2266 (Boerhinger Ingelheim Co.), and naloxone (DuPont Pharmaceutical Co.), representing k- and μ-antagonists, respectively. Their affinities to three types of opioid receptors are reflected from their binding selectivities to these receptors in the guinea-pig brain membrane as shown in Table 2. It is obvious that DAGO, U50,488H and DPDPE are rather specific agonists for μ-, k- and δ-receptors, respectively. DADLE has a relatively higher affinity to δ-receptors with an affinity to μ-receptors about half that of δ-receptors. MR 2266 and naloxone are predominantly k- and μ-antagonists, respectively.

Except MR 2266 which was dissolved in dimethyl sulfoxide (DMSO), all drugs were dissolved in Krebs–Ringer solution. The agonists and antagonists were infused at volumes of 20 and 5 μl/min, respectively by separate cannulae leading directly into the aorta. Except U50,488H which was aministered at a dose range of 15, 44 and 132 nmol/heart, the doses administered were 44, 132 and 396 nmol/heart for other agonists. Antagonists were injected 1 min before the administration of agonist or ischemia. The dose used in the former case was 30 nmol/heart while those used in the latter case were 38, 114 and 342 nmol/heart. In deciding the dose range of opioid agonists, various doses of U50,488H were tried first based on our previous studies (Wong and Lee, 1987; Lee and Wong, 1987b) reporting the arrhythmogenic effect of dynorphin$_{1-13}$, a selective k-agonist (Chavkin *et al.*, 1982). Two criteria were used (1) it induced arrhythmias and (2) the effect was antagonized by specific antagonists as an indication that the effect of the agonists was a result of activation of specific opioid receptors. A similar but slightly higher dose range was used for other agonists to enable comparison. The choice of dose of antagonists was also based on (1) the results of the previous studies (Wong and Lee, 1987; Lee and Wong, 1986; 1987b) and (2) trial and error as we did in the determination of dose range for agonists. An important criterion was that the antagonists themselves did not produce any effect on cardiac rhythm.

Statistical analysis

Chi-square test and Student's *t*-test were employed to analyse the difference in the incidence or arrhythmias and arrhythmia score between groups, respectively.

TABLE 1. An arrhythmia scoring system

Arrhythmia score	Type of arrhythmia
0	no arrhythmia
1	atrial arrhythmias or occasional PVC
2	frequent PVC
3	VT (1–2 episodes)
4	VT (> 3 episodes) or VF (1–2 episodes)

TABLE 2. Binding selectivity profile of agonists and antagonists in guinea-pig brain membrane

Ligand	Ki (nM) μ	k		Reference
Agonists				
DAGO	1.9	6090	345	Corbett *et al.*, 1984
U50,488H	941	0.72	8690	James and Goldstein, 1984
DPDPE	710	>15,000	2.7	Corbett *et al.*, 1984
DADLE	3.2	9600	1.5	James and Goldstein, 1984
Antagonists				
MR 2266	1.37	0.69	6.0	Magnan *et al.*, 1982
Naloxone	1.78	17.2	27.0	Magnan *et al.*, 1982

Results

Effects of opioid agonists on cardiac rhythm in the isolated perfused rat heart (Table 3 and Fig 1)

DAGO, the selective μ-agonist, at doses of 44, 132 and 396 nmol/heart induced atrial arrhythmias in 2, 5 and 7 out of 8 hearts, respectively. The incidence of atrial arrhythmias at the doses of 132 and 396 nmol/heart was statistically significant compared with the control. The peptide at the dose of 396 nmol/heart caused frequent PVC in three out of eight hearts with 1 exhibiting VT as well. The effect of the peptide was dose-related. U50,488H, the selective k-agonist, at the doses, 44 and 132 nmol/heart, caused both atrial and ventricular arrhythmias. 6 and 2 out of 8 hearts that received 44 nmol/heart of the drug exhibited atrial arrhythmias and frequent PVC, respectively. At a higher dose, 132 nmol/heart, U50,488H induced malignant types of ventricular arrhythmias, namely frequent PVC and VT, in at least half of the hearts-6 and 4 in the groups without or with DMSO. The effects of the drug were also dose related and the difference in incidence of arrhythmias was statistically higher than that of the control group. At the dose of 132 nmol/heart, U50,488H induced either frequent VPC or VT in 6 out of 8 hearts, whereas DAGO only induced atrial arrhythmias in five out of eight hearts, an effect similar in severity to that of U50,488H at the dose of 15 nmol/heart, 1/9 of its dose. The arrhythmia score for the group receiving U50,488H at the dose of 132 nmol/heart was significantly higher than the corresponding value in the group receiving the same dose of DAGO [Fig. 1(a)]. On the other hand, the effects of the -agonists, DPDPE and DADLE on cardiac rhythm seemed to be negligible. Injection of DPDPE or DADLE at a dose of 396 nmol/heart caused either atrial arrhythmias-PAC and A–V block or occasional PVC in three or two hearts out of eight and six hearts, respectively. The arrhythmias were not severe at all nor was the difference in incidence of arrhythmia statistically significant from that of the control.

The arrhythmogenic effects of U50,488H or DAGO were attenuated by pretreatment of MR 2266 or naloxone, respectively as indicated by significant reductions in incidence of arrhythmias (Table 3) and arrhythmia score [Fig. 1(b)].

Effects of k- and μ-antagonists against arrhythmias arising during ischemia and reperfusion (Table 4 and Fig. 2)

MR 2266 at doses of 38, 114 and 342 nmol/heart significantly reduced the incidence of arrhythmia in a dose related manner during ischemia and reperfusion. The onset of arrhythmia during ischemia was also significantly delayed. After administration of MR 2266 at the dose of 38 nmol/heart, the malignant types of ventricular arrhythmias were almost abolished during reperfusion- only one out of eight hearts exhibit frequent PVC, significantly lower than the control group in which all eight hearts exhibited either frequent PVC or VT/VF or both. MR 2266 at the dose of 114 and 342 nmol/heart almost

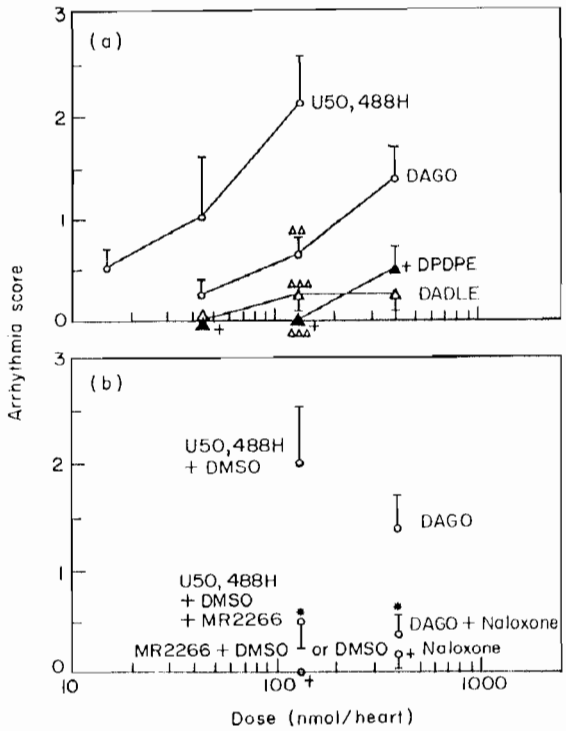

FIGURE 1. Arrhythmia scores after administration of opioid agonists and/or antagonists in the isolated perfused rat heart (a) Opioid agonists; (b) Opioid antagonists alone or before opioid agonists.

Values are Mean ± S.E.M; $n = 8$ in all groups except in those with + in which $n = 6$

*significantly lower than the corresponding group without pretreatment of antagonist to the level $P < 0.05$.

†, ‡ significantly lower than the corresponding group receiving a same dose (132 nmol/heart) of U50,488H to the levels $P < 0.01$ and 0.001, respectively.

completely abolished all kinds of arrhythmias during reperfusion with only one out of eight heats in the group receiving 342 nmol/heart of U50,488H exhibiting occasional PAC. The arrhythmia scores were significantly lower than that of the control. On the other hand, pretreatment of naloxone at the same dose range did not delay the onset of arrhythmia during ischemia. Unlike the hearts pretreated with MR 2266, frequent PVC still persisted during reperfusion; the incidences were higher than the corresponding values after administration of MR 2266. Similar to MR 2266, naloxone also abolished all the VT during reperfusion and its effects were dose-dependent. The arrhythmia scores were significantly higher than the corresponding values in the MR 2266-treated groups except at the dose of 342 nmol/heart.

Discussion

It was found in the present study that U50, 488H has by far the most potent arrhythmogenic effects among the three prototypic opioid agonists in the isolated perfused rat heart preparation as evidenced by 1, its ability in inducing the more severe type of ventricular arrhythmias such as VT at doses, 44 and 132 nmol/heart at which other agonists either produced negligible effects or predominantly atrial arrhythmias (Table 3) and 2, higher arrhythmias score [Fig. 1(a)]. The results are in agreement with our previous findings that dynorphin$_{1-13}$, another selective k-receptors agonist, induces ventricular arrhythmias (Wong and Lee, 1987) while β-endorphin, an agonist with high affinities to μ- and δ-receptors (Kosterlitz *et al.*, 1986), causes atrial arrhythmias only (Lee *et al.*, 1984) in the isolated rat heart. The findings indicate that

TABLE 3. Effects of DAGO, U50,488H, DDDPE and DADLE (in nmol/heart) on cardiac rhythm in the isolated perfused rat heart

	N	Atrial arrhythmias	Ventricular arrhythmias					
			PVC		VT			Total of FPVC +VT
			O	F	Incidence	Episode	Duration (Sec.)	
Control	8	0	0	0	0	0	0	0
DAGO 44	8	2	0	0	0	0	0	0
DAGO 132	8	5*	0	0	0	0	0	0
DAGO 396	8	7***	0	3	1	1	20	3
DAGO 396 + Naloxone	8	3†	1	0	0	0	0	0
Naloxone only	6	1	0	0	0	0	0	0
U50,488H 15	8	4*	0	0	0	0	0	0
U50,488H 44	8	6**	2	2	0	0	0	2
U50,488H 132	8	7***	0	5*	3	3.0 ± 1.53	19.3 ± 9.25	6**
U50,488H 132 with DMSO	8	6	5	2	3	3.33 ± 1.45	6.33 ± 3.85	4
U50,488H 132 + MR 2266 in DMSO	8	1†	1†	1	0	0	0	1
MR 2266 in DMSO	6	0	0	0	0	0	0	0
DMSO only	6	0	0	0	0	0	0	0
DADLE 44	8	0	0	0	0	0	0	0
DADLE 132	8	2	0	0	0	0	0	0
DADLE 396	8	1	1	0	0	0	0	0
DPDPE 44	6	0	0	0	0	0	0	0
DPDPE 132	6	0	0	0	0	0	0	0
DPDPE 396	6	2	1	0	0	0	0	0

Values in episode and duration of VT are mean ± S.E.M.; Doses of antagonists used were 30 nmol/heart.
*, **, *** Statistically different from the control without administration of opioid agonist at levels $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
†, Statistically different from the corresponding group without pretreatment of opioid antagonist at the level $P < 0.05$.
PVC: Premature Ventricular Contraction; VT: Ventricular Tachycardia; F: Frequent; O: Occasional.

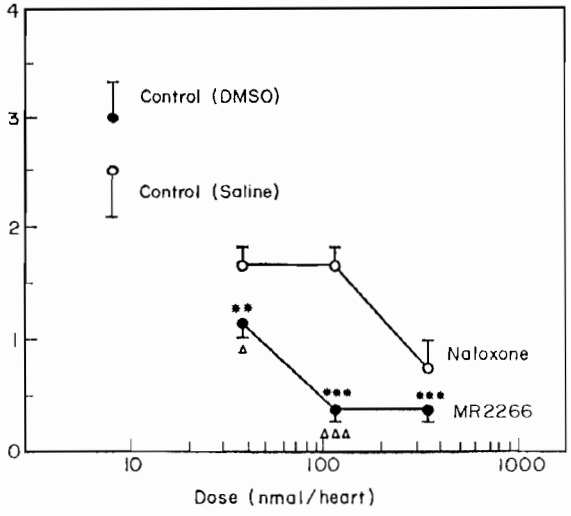

FIGURE 2. Arrhythmia scores during ischemia and reperfusion after administration of opioid antagonists. Values are Mean ± S.E.M; $n = 8$ in all groups. **,*** significantly lower than the control at levels $P < 0.01$ and $P < 0.001$, respectively. †, ‡ significantly lower than the corresponding groups receiving naloxone at the levels $P < 0.05$ and $P < 0.001$, respectively.

k-agonists are the most potent in induction of cardiac arrhythmias. The effects resulted from interacting with the k-receptors as they were attenuated by MR 2266, a specific k-antagonist [Table 3 and Fig. 1(b)].

In the present study it was also found that there are two notable differences between these two antagonists in their effects against cardiac arrhythmias arising during ischemia and reperfusion. Firstly, the onset of arrhythmias during ischemia after pre-treatment with MR 2266 was significantly delayed. Secondly, the incidence of arrhythmias particularly frequent PVC during reperfusion in the groups pre-treated with MR 2266 at doses 38 and 114 nmol/heart was lower than those of the corresponding group pretreated with same doses of naloxone. As a consequence the arrhythmia scores after administration of naloxone were higher than the corresponding values after administration of the same doses of MR 2266. The results indicate that MR 2266, which has a relatively high affinity to k-receptors, is more effective than naloxone, which binds with u-receptors with the highest affinity, in antagonising arrhythmias arising during ischemia and reperfusion (Table 4 and Fig. 2). In agreement with the finding, Sitsapesan and Parratt (1989) found that in the anesthetised rat MR 2266 is the most potent antiarrhythmic agent among the three types of opioid antagonists during ischemia.

It has been shown that in the heart are dynorphins or dynorphin- like peptides (Weihe *et al.*, 1985), which are predominantly k-agonists, and enkephalins or enkephalin-containing peptides (Lang *et al.*, 1983; Weihe *et al.*, 1983; 1985), which are predominantly δ-agonists. Together with the findings of the present study that k-agonist, U50,488H, is most potent in induction of cardiac arrhythmias and δ-agonists are almost non- arrhythmogenic and that k-antagonist, MR 2266 is more antiarrhythmic than the μ-antagonist, naloxone, against arrhythmias during ischemia and reperfusion, it is obvious that k-receptors are the likely receptor-subtype that is involved in arrhythmogenesis during ischemia and reperfusion. Further studies are needed to identify the opioid peptide(s) that is(are) released during ischemia and reperfusion and to elucidate the events that occur following the activation of cardiac opioid receptors.

TABLE 4. Effects of MR2266 or naloxone (in nmol/heart) against arrhythmias arising during ischemia and reperfusion

		Ischemia					Reperfusion							
				Ventricular arrhthmias PVC					Ventricular Arrhythmias					
									PVC		VT			Total
	N	Onset (min)	Atrial arrhythmias	O	F	Total	Onset (min)	Atrial arrhythmias	O	F	Incidence	Episode	VF	of FPVC, VT & VF
Control DMSO	8	2.88 ± 0.74	8	1	0	8	1.75 ± 0.53	8	2	6	5	3.80 ± 1.24	1	8
MR 2266 38 DMSO	8	12.1 ± 2.65**	6	1	0	6	1 ± 0	8	3	1**	0**	—	0	1***
MR 2266 114 DMSO	8	10.7 ± 1.77**	3*	0	0	3*	—	0***	0	0**	0*	—	0	0***
MR 2266 342 DMSO	8	10.5 ± 0.5**	2**	0	0	2**	0.5	1***	0	0**	0*	—	0	0***
Control	8	4.63 ± 1.19	8	0	0	8	1.38 ± 0.26	8	2	6	4	1.75 ± 0.75	1	6
Naloxone 38	8	2.56 ± 0.88‡	8	0	1	8	1.06 ± 0.15	6	3	4	0*	—	0	4
Naloxone 114	8	5.60 ± 1.91	5	0	0	5	1.33 ± 0.25	3*	3	5†	0*	—	0	5†
Naloxone 342	8	10.5 ± 5.0	2**	0	0	2**	1.0 ± 0	0***	3	1**	0*	—	0	1**

Values in onset of arrhythmias and episode of VT are mean ± S.E.M.
*,**,*** statistically different from the corresponding control at the levels $P < 0.005$, $P < 0.01$ and $P < 0.001$, respectively
†, ‡ statistically different from the corresponding groups receiving same doses of MR 2266 at the levels $P < 0.05$ and $P < 0.01$, respectively.
Abbreviation: see Table 2.

Acknowledgement

We thank Dr J. C. L. Wong for the helpful discussion, Mr C. P. Mok for technical assistance, Mrs Cindy Li for preparation of the manuscript and Mr R. K. W. Chan for drawing the figures, respectively. The study was supported by the Croucher Foundation. U50,488H, MR 2266 and naloxone were kindly supplied by the Upjohn Co., Boehringer Ingelheim Co. and DuPont Pharmaceutical Co., respectively.

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