J. Pharm. Pharmacol. 1995, 47: 52–58
Received November 9, 1993
Accepted September 7, 1994

The Effects of Opioid Peptides on Cardiovascular Function and Sympathetic Neurotransmission in Rats

M. A. MCINTOSH, K. A. KANE AND J. R. PARRATT

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, UK

Abstract

The haemodynamic effects of three opioid receptor agonists, with some preferential activity on δ-, μ- and κ-receptors were investigated in anaesthetized and pithed rats, and effects on sympathetic neurotransmission were also investigated in pithed rats.

In anaesthetized rats, D-Ala^2-D-Leu^5-enkephalin (DADLE) (a predominantly δ-receptor agonist, 10 μg kg^{-1}), glyol (μ, 0·5 mg kg^{-1}) and *R,S-N*-C2-*N*-methyl-3,4,dichloro-phenylacetamido-2-C3-carboxy phenylethyl-pyrrolidine (ICI 204448) (κ, 0·1 mg kg^{-1}) by intravenous administration transiently decreased heart rate from 462 ± 12 to 432 ± 14, 460 ± 12 to 448 ± 13 and 460 ± 12 to 448 ± 11 beats min^{-1}, respectively, and mean arterial blood pressure from 142 ± 6 to 111 ± 9, 141 ± 6 to 122 ± 5 and 148 ± 7 to 121 ± 6 mmHg, respectively. The effects of DADLE, but not those of glyol or ICI 204448, were blocked by M8008, a δ-receptor antagonist. In pithed rats, none of the opioid agonists had any significant effects on heart rate or mean arterial blood pressure; however, acetylcholine significantly reduced both heart rate and mean arterial blood pressure. All three opioid agonists reduced the positive chronotropic response to thoracic (C7-T2) spinal cord stimulation in pithed rats, by 17 ± 4, 30 ± 2 and 20 ± 10% for DADLE, glyol and ICI 204448, respectively. This compared with a 48 ± 15% reduction with clonidine (5 μg kg^{-1}). This effect of DADLE was almost abolished by M8008.

It is concluded that the haemodynamic effects of the opioid agonists studied are mediated via actions on the central nervous system and that a decrease in sympathetic neurotransmission may account for, at least in part, the bradycardia produced by opioid agonists in intact anaesthetized rats. It seems that the sympathetic nervous system is unlikely to be involved in the arrhythmogenic effects of opioid peptides.

It has been proposed that endogenous opioid peptides play a role in cardiac arrhythmogenesis during acute myocardial ischaemia (Fagbemi et al 1982; Lee et al 1984; Parratt & Sitsapesan 1986). The mechanism by which opioid peptides may exert an arrhythmogenic effect has been little studied. It is known that opioid peptides and opioid receptors are located in the heart (Lang et al 1983; Weihe et al 1983, 1985) and vasculature (Sun & Zhang 1985). Opioid peptides and opiate alkaloids exert marked cardiovascular responses in-vitro (Clo et al 1985). In addition to direct postsynaptic effects on cardiac (Vargish et al 1987) and vascular smooth muscle (Altura et al 1984), opioid peptides may alter cardiovascular function by acting presynaptically to modulate sympathetic neurotransmission (Illes et al 1983; Starke et al 1985). However, most of the experiments examining the effects of the opioid peptides on sympathetic neurotransmission have been performed in-vitro. It is thought that, in-vivo, an increase in sympathetic nerve activity is one important factor in the development of early ventricular arrhythmias during acute myocardial ischaemia (Schomig & Richardt 1991), and it is possible that the sympathetic nervous system may be involved in the production of arrhythmias by endogenous opioid peptides.

The aim of the present study was to investigate in-vivo the effects of three opioid agonists on peripheral sympathetic neurotransmission in pithed rats. Furthermore, the effects of these opioid agonists on heart rate and arterial blood pressure were examined in both pithed and anaesthetized rats. The opioid agonists studied were DADLE (a ligand at δ-receptors) (Gillan & Kosterlitz 1982), glyol (mainly μ-receptor selective) (Handa et al 1981; Kosterlitz & Paterson 1981) and ICI 204448 (a peripherally acting κ-agonist) (Shaw et al 1989). Agonists acting at the three main opioid receptor subtypes were chosen for investigation because it has been reported that agonists with affinity for μ- and δ- (Lee et al 1984) and κ-receptors are arrhythmogenic in-vitro (Wong et al 1990). Although it was not the aim of this study to identify the receptor subtypes involved in the observed responses, the effects of one opioid receptor antagonist, M8008 (16-methyl cyprenorphine) which is more potent on δ- and μ-opioid receptors (Smith 1987), on the responses to the above agonists, were also investigated. This antagonist was examined because it had been shown to be antiarrhythmic in anaesthetized rats subjected to coronary artery occlusion (McIntosh et al 1992). This work has been presented previously in abstract form (McIntosh et al 1991).

Correspondence: K. A. Kane, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, UK.

Materials and Methods

Anaesthetized rats

Anaesthesia was induced in male Sprague-Dawley rats, 250–300 g, with sodium pentobarbitone, 60 mg kg^{-1}, given intraperitoneally. The trachea was cannulated for artificial respiration and the femoral vein for drug administration. Systemic blood pressure was monitored from the carotid artery. A standard lead I ECG was recorded together with systemic blood pressure using a Mingograf 82 ink jet recorder (Elema-Schonander, Stockholm). Animals were then respirated with room air using a Bioscience (UK)

respirator (volume 1·5 mL/100 g, rate 54 strokes min^{-1}). The three opioid agonists were given intravenously as consecutive bolus injections following full recovery from the previous injection. In preliminary experiments it was established by varying the order of administration of the agonists, that there was no apparent cross-tolerance to the cardiovascular effects of the drugs studied. In a different set of experiments, the opioid agonists were given 5 min after the intravenous bolus injection of the δ-preferring opioid antagonist, M8008.

Pithed rats

The procedure used was that described by Gillespie et al (1970). Male Sprague-Dawley rats, 250–300 g, were anaesthetized with ether and the trachea intubated. Rats were pithed by inserting a short steel tube (13 swg) through the orbit of the eye and down the spinal column via the foramen magnum. Through this trocar was then passed a Teflon tube (1·5 mm diam.) and inside this a fine steel rod (26 swg) which was pushed to the sacral end to complete the pithing. An indifferent steel electrode was inserted under the skin of the back of the animal, parallel to the spinal column. Rats were immediately artificially ventilated with 100% O_2 at a rate of 54 strokes min^{-1} and a volume 1 mL/100 g. A cannula was placed in the right femoral vein, or the left ventricle by way of the right common carotid artery, to allow for intravenous or left intraventricular drug administration. The right femoral artery was also cannulated for the measurement of arterial blood pressure. Heart rate was assessed from the blood pressure trace or from the electrocardiogram.

The central steel rod used for pithing served as the stimulating electrode. This rod was positioned within the vertebral canal to allow electrical stimulation of the thoracic spinal cord (C7-T2). The animals were left to equilibrate for 30–45 min and 15 min before starting the electrical stimulation, vecuronium (bolus of 1 mg kg^{-1} and then a continuous infusion 160 μg kg^{-1} min^{-1}) was administered to reduce skeletal muscle movements. The spinal cord (C7-T2) was stimulated continuously (0·1 to 0·2 Hz, 0·5 ms, 50 V) for a further 10 min. When the stimulation-induced heart rate response had reached a plateau (normally after 5 min) either saline, clonidine (to test for presynaptic inhibition) or the opioid agonists were administered as bolus injections. Only one drug was examined in each animal. In a different set of experiments, the effects of each of the agonists on basal heart rate and blood pressure were also determined.

In a separate set of experiments, and using a protocol similar to that described above, the effects of DADLE were investigated in the presence of M8008. DADLE was given at the fifth minute of spinal cord stimulation and at the seventh minute post-stimulation, when the response to DADLE was stable, M8008 was administered intravenously. The changes in heart rate and arterial blood pressure in the absence and presence of M8008 were compared.

Drugs

DADLE (D-Ala^2-D-Leu^5-enkephalin), glyol (Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH), clonidine hydrochloride and acetylcholine chloride were obtained from Sigma (Dorset). ICI 204448 (*R,S-N*-C2-*N*-methyl-3,4, dichloro-phenylacetamido-2-C3-carboxyphenylethyl-pyrrolidine) was from Cambridge Research Biochemicals (Cheshire) and vecuronium was from Organon Ltd (Lanarkshire) M8008 (16-methyl cyprenorphine hydrochloride) was a gift from Reckitt & Colman (Hull).

The drugs were dissolved in 0·9% w/v NaCl, with the exception of vecuronium, which was dissolved in aqueous citric acid. A stock solution of ICI 204448 was prepared in distilled water with the aid of a few drops of 0·1 M NaOH; the pH was then adjusted to neutral with 0·1 M HCl.

Statistics

Data are given as mean ± s.e.m. In all comparisons, unpaired or paired Student's *t*-tests were employed as appropriate. $P < 0.05$ was considered to be statistically significant.

Results

Anaesthetized rat

Preliminary experiments were carried out in anaesthetized rats to assess the effects on heart rate and arterial blood pressure of a range of concentrations of the three opioid agonists studied. DADLE, given as bolus intravenous injections of 5, 10, 25, 50 and 500 μg kg^{-1} caused a transient fall in heart rate and mean arterial blood pressure. The maximum reduction in each of these variables was observed with 50 μg kg^{-1}, with falls in heart rate and mean arterial pressure of 84 beats min^{-1} and 63 mmHg, respectively. Further experiments were carried out with either 10 μg kg^{-1}, a dose which produced about 50% of the maximal response (i.e. falls in heart rate and mean arterial pressure of 45 beats min^{-1} and 36 mmHg, respectively) or, in some of the experiments in pithed animals with 500 μg kg^{-1} (a maximally effective dose). Glyol, in doses of 0·25, 0·5, 2·5, 250 μg kg^{-1} and 2·5 mg kg^{-1} also caused transient reductions in heart rate and mean arterial blood pressure with maximal reductions in heart rate and mean arterial pressure of the order of 50 beats min^{-1} and 65 mmHg, respectively. Doses of 0·5 μg kg^{-1} (falls in heart rate of 11 beats min^{-1} and in arterial pressure of 18 mmHg) and 2·5 mg kg^{-1} were selected for further experiments as these caused a submaximal and a maximal effect, respectively. The lowest dose of ICI 204448 studied, 1 μg kg^{-1}, had no effect on either heart rate or arterial blood pressure, but doses of 0·1, 1 and 5 mg kg^{-1} caused a similar reduction in heart rate (of the order of 12 beats min^{-1}), but a concentration-dependent fall in arterial blood pressure (with a maximal fall in pressure of 32 mmHg). In further experiments a dose of 0·1 mg kg^{-1} ICI 204448 was used.

The reductions in heart rate and mean arterial blood pressure produced by DADLE (10 μg kg^{-1}), glyol (0·5 μg kg^{-1}) and ICI 204448 (0.1 mg kg^{-1}) in anaesthetized rats are shown in Fig. 1. All three opioid agonists reduced heart rate within 30 s of administration and recovery was complete 3 min after administration. Administration of an equivalent volume of saline was without effect. This fall in heart rate was accompanied by a reduction in mean arterial blood pressure. The effect on blood pressure of the relatively κ-selective agonist, ICI 204448, was more prolonged than that of either DADLE or glyol.

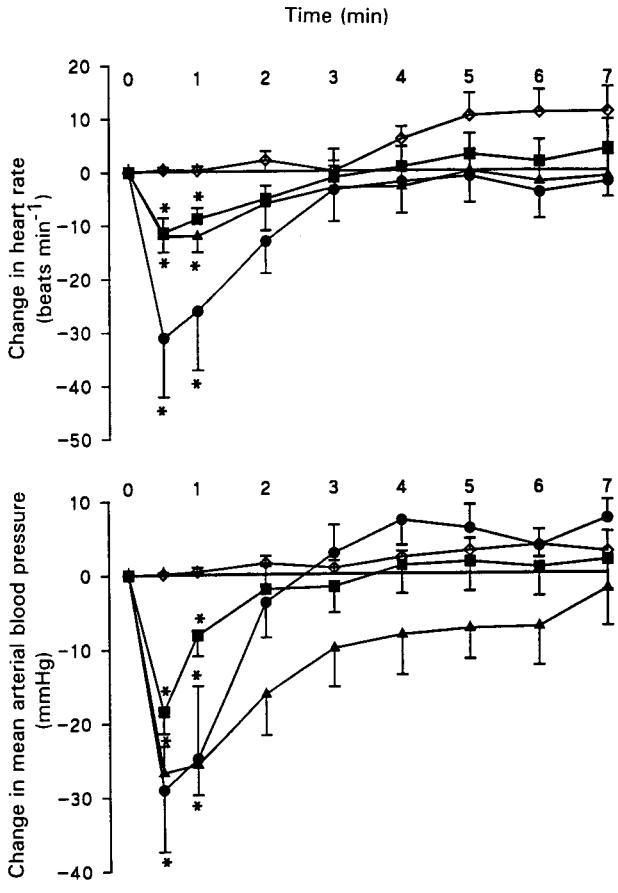

FIG. 1. The change in heart rate and mean arterial blood pressure produced by DADLE (10 μg kg^{-1}, ●), glyol (0·5 μg kg^{-1}, ■) and ICI 204448 (0·1 mg kg^{-1}, ▲), in anaesthetized rats. Saline controls are also shown (◇). Values are presented as mean ± s.e.m., n = 7, *$P < 0·05$ compared with pre-drug values. Basal heart rate values were 462 ± 12, 460 ± 12 and 460 ± 12 beats min^{-1} in the DADLE-, glyol- and ICI 204448-treated groups, respectively. Basal mean arterial blood pressure values were 142 ± 6, 141 ± 6 and 148 ± 7 mmHg in the DADLE-, glyol- and ICI 204448-treated groups, respectively.

The effects of M8008 in a dose of 0·5 mg kg^{-1} were studied because our previous work in rats subjected to coronary artery occlusion had shown that at this dose, M8008 possessed marked antiarrhythmic activity (McIntosh et al 1992). At this concentration M8008 had no effect on either mean arterial blood pressure or heart rate before the administration of the opioid receptor agonists. Values for mean arterial blood pressure and heart rate were 126 ± 4 vs 124 ± 5 mmHg and 433 ± 12 vs 440 ± 14 beats min^{-1} before and at 5 min post-drug, respectively.

The effects of the three opioid agonists studied, in the presence of M8008, on arterial blood pressure and heart rate are summarized in Table 1. In the presence of M8008, DADLE (10 μg kg^{-1}) had no effect on arterial blood pressure or heart rate whereas the haemodynamic effects of glyol and ICI 204448 were similar to those observed in the absence of M8008 (see Fig. 1).

Pithed rat

Basal heart rate and blood pressure. Basal heart rate and arterial blood pressure were significantly lower in pithed rats than in anaesthetized rats. Typical control values for heart rate and mean arterial blood pressure, were 273 ± 3 beats min^{-1} and 54 ± 1 mmHg (n = 40), respectively, compared with 444 ± 11 beats min^{-1} and 98 ± 4 mmHg (n = 23) in anaesthetized animals.

Table 1. Effects of DADLE (10 μg kg^{-1}, n = 6), glyol (0·5 μg kg^{-1}, n = 6) and ICI 204448 (0·1 mg kg^{-1}, n = 6) in the presence of M8008, 0·5 mg kg^{-1}, on mean arterial blood pressure (MABP) and heart rate before and at 30 s and 3 min posttreatment in anaesthetized rats.

Treatment	Pre-treatment	Post-treatment 30 s	Post-treatment 3 min
MABP (mmHg)			
DADLE	128 ± 11	124 ± 11	127 ± 10
Glyol	125 ± 6	113 ± 6*	132 ± 7
ICI 204448	132 ± 7	97 ± 4*	112 ± 6
Heart rate (beats min^{-1})			
DADLE	445 ± 18	438 ± 19	441 ± 18
Glyol	440 ± 12	432 ± 16	442 ± 15
ICI 204448	446 ± 12	432 ± 10*	432 ± 8*

* Denotes significantly different from pretreatment values.

Neither DADLE nor glyol, in concentrations up to 0·5 and 2·5 mg kg^{-1}, respectively, had any significant effect on basal cardiovascular variables in pithed rats when administered intravenously or directly into the left ventricle. For example, respective heart rates and blood pressures were 286 ± 3 vs 286 ± 3 beats min^{-1} and 61 ± 4 vs 65 ± 7 mmHg (n = 4) before and at 1 min post-intravenous administration of DADLE (0·5 mg kg^{-1}). Before and after intraventricular administration of the same dose of DADLE, the values were 288 ± 10 vs 286 ± 9 beats min^{-1} and 58 ± 4 vs 60 ± 5 mmHg (n = 4). ICI 204448, at 0·1 mg kg^{-1}, did not significantly reduce heart rate (values of 326 ± 18 and 303 ± 18 beats min^{-1} pre- and post-drug, respectively), or arterial blood pressure (61 ± 4 vs 62 ± 4 mmHg; n = 4 at 1 min post-intravenous administration).

This failure of opioid agonists to alter heart rate and arterial blood pressure in pithed animals is unlikely to be due to the low basal values since acetylcholine (10 μg kg^{-1}) given intravenously was able to significantly reduce both heart rate (from 289 ± 12 to 259 ± 12 beats min^{-1}) and mean arterial blood pressure (from 51 ± 2 to 30 ± 2 mmHg, n = 11). These chronotropic and vasodepressor responses to acetylcholine were transient and lasted approximately 15–30 s.

Electrical stimulation of the spinal cord (C7-T2). Electrical stimulation of sympathetic outflow from the C7-T2 region of the spinal cord causes statistically significant increases in heart rate and arterial blood pressure. The cardio-accelerator response peaked at around 4 min (+73 ± 2 beats min^{-1}, n = 7) and was maintained throughout the remaining stimulation period (Fig. 2). The stimulation-induced pressor response (+11·9 ± 1·9 mmHg) was maximal within 1 min of stimulation. Thereafter, a time-related decline in arterial blood pressure occurred despite continued electrical stimulation (Fig. 2). Fig. 2 also shows that administration of saline did not modify the stimulation-induced cardio-accelerator or vasopressor responses when administered

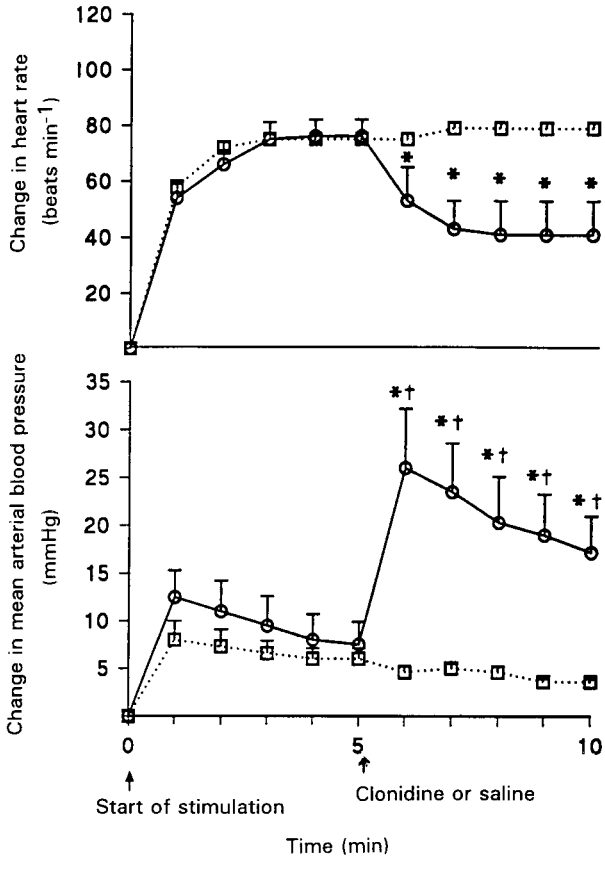

FIG. 2. The actions of clonidine (5 μg kg^{-1}, ○, n = 4) and saline (□, n = 4), given intravenously, on the cardio-accelerator response (heart rate) and the increase in mean arterial blood pressure to sympathetic (C7-T2) nerve stimulation in pithed rats. Values are given as mean ± s.e.m. *$P < 0.05$ compared with the pre-drug value; $^{+}P < 0.05$ compared with the control saline group. Pre-stimulation values for heart rate and mean arterial blood pressure were 281 ± 22 beats min^{-1} and 56 ± 4 mmHg, respectively, in the saline-treated group and 312 ± 33 beats min^{-1} and 54 ± 3 mmHg, respectively, in the clonidine-treated group.

5 min after commencing the spinal cord stimulation. Clonidine (5 μg kg^{-1}) significantly reduced the positive chronotropic response (a maximum reduction of 48 ± 15% after 3 min) and increased the mean arterial blood pressure with a peak effect at 1 min post-administration (Fig. 2).

DADLE (10 μg kg^{-1}), given intravenously, tended to reduce the heart rate response to electrical stimulation (from +114 ± 15 to +106 ± 18 beats min^{-1}) but tended to have no effect on the vasopressor response (+6·5 ± 4·9 vs +9·5 ± 4·2 mmHg at 7 min of stimulation i.e. 2 min post-drug administration). Fig. 3 demonstrates the effects of DADLE, at a higher dose (0·5 mg kg^{-1}), on the increase in heart rate and arterial blood pressure produced by sympathetic nerve stimulation. At this dose, DADLE, administered intravenously, caused statistically significant reductions in the stimulation-induced cardio-acceleration (Fig. 3). Like clonidine, this inhibitory response of DADLE (17 ± 4% reduction) peaked at 2–3 min and was sustained for the remaining observation period. A similar, but not significant, effect on the cardio-acceleratory response to electrical stimulation (15 ± 6% reduction) was observed when DADLE was given intraventricularly. DADLE (0·5 mg kg^{-1}) had no significant effect on the arterial blood pressure during spinal cord stimulation although, following both intravenous and intraventricular administration, arterial blood pressure declined more rapidly than in saline-treated rats. Since the route of administration did not influence the results obtained with DADLE, subsequent experiments were performed with intravenous administration only.

M8008 (0·5 mg kg^{-1}) reversed the DADLE-induced inhibitory effect on the cardio-acceleration response in pithed rats (Fig. 3). In animals which received both DADLE and M8008, stimulation of the spinal cord increased heart rate by 100 ± 3 beats min^{-1} before administration of drugs. DADLE alone reduced significantly this chronotropic response to 78 ± 2 beats min^{-1} and by 3 min after administration of M8008 this inhibitory effect was almost abolished and the chronotropic response increased to 94 ± 3 beats min^{-1} (n = 5). M8008 also prevented the more rapid fall in arterial blood pressure produced by DADLE (Fig. 3).

Glyol, in a dose of 0·5 μg kg^{-1}, had no significant effects on the cardio-acceleration induced by sympathetic nerve (C7-T2) stimulation. There was an increase in heart rate of 97 ± 16 beats min^{-1} both at 5 min (i.e. pre-drug) and 7 min (i.e. 2 min post-drug) of spinal cord stimulation (n = 4). However, in a higher dose (2·5 mg kg^{-1}), glyol significantly reduced (by 30 ± 2%) the positive chronotropic response to sympathetic nerve stimulation although it had no effect on the arterial blood pressure (Fig. 4). ICI 204448 (0.1 mg kg^{-1}) also reduced the stimulation-induced tachycardia (by 20 ± 10%) but exerted no significant effect on the pressor response.

Discussion

The opioid receptor agonists DADLE (δ), glyol (μ) and ICI 204448 (κ) all decreased heart rate and blood pressure in intact anaesthetized rats but had no significant effects on basal haemodynamic parameters in pithed rats. This lack of effect in pithed rats is unlikely to be due to the much lower control values of heart rate and blood pressure in these animals since it was possible to observe both a negative chronotropic and a vasodepressor effect in this preparation with acetylcholine. The results obtained with the opioid agonists suggest that the cardiovascular effects may, at least in part, be centrally mediated or may require an intact autonomic nervous system. ICI 204448 (Shaw et al 1989) and glyol (Zimmerman & Leander 1990) cross the blood-brain barrier very poorly, indicating that a central basis for their action is unlikely. Furthermore, ICI 204448 tended to cause bradycardia in the pithed rat, a finding which has been reported in this model for other κ-receptor agonists such as ethylketocyclazocine (Gautret & Schmitt 1984) and dynorphin(1–13) (Gautret & Schmitt 1985). These responses were prevented by the κ-opioid receptor-preferring antagonist Mr 2266 Bs (Romer et al 1980), suggesting that the bradycardia produced by ethylketocyclazocine, dynorphin and perhaps ICI 204448 is mediated by an action at post-synaptic κ-sites located directly on the heart.

In addition, and in a similar manner to clonidine, all three opioid agonists reduced the cardiac acceleratory response to

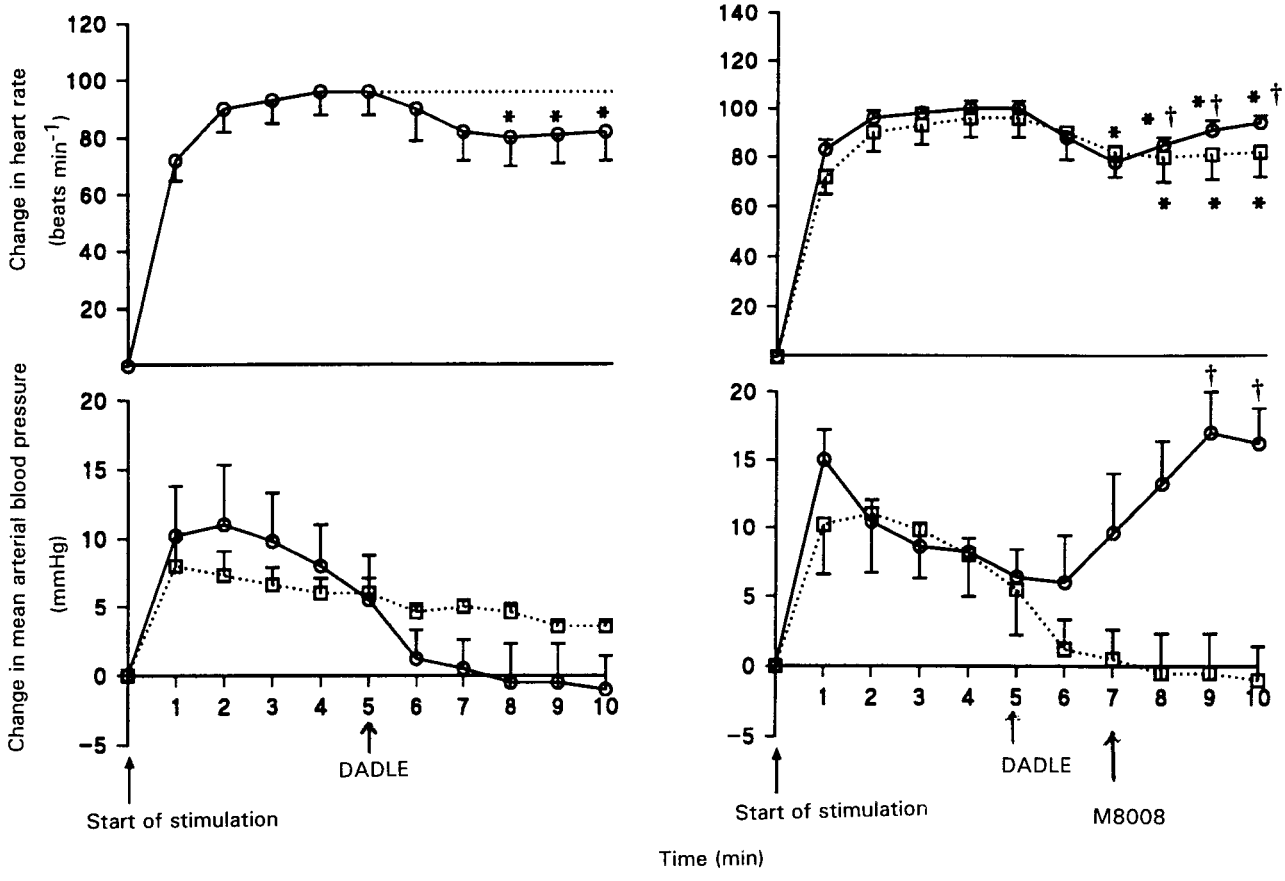

FIG. 3. The effects of DADLE (0.5 mg kg^{-1}, n = 4), given intravenously either in the absence (left hand panel) or presence (right hand panel) of M8008 (0.5 mg kg^{-1}), on the heart rate increase and the vasopressor response produced by nerve stimulation in pithed rats (○). The dotted line illustrates the saline control group. Values are given as mean ± s.e.m.; *$P < 0.05$ compared with the pre-drug value. Pre-stimulation values for heart rate and mean arterial blood pressure were 226 ± 2 beats min^{-1} and 57 ± 4 mmHg, respectively, in the DADLE-treated group.

sympathetic nerve stimulation in pithed rats. The clonidine-induced reduction of the positive chronotropic response to electrical stimulation of the spinal cord results from a decrease in noradrenaline release, following activation of presynaptic α-adrenoceptors located on the nerve endings (Docherty & McGrath 1980). Thus, the opioid agonists may also act presynaptically to inhibit sympathetic neurotransmitter release in the heart. In accord with this suggestion, there have been reports which demonstrate that a number of opioid peptides and opiates can inhibit, presynaptically, the cardiac responses to sympathetic nerve stimulation under in-vitro conditions. For instance, in the rabbit isolated heart, the κ-receptor agonist ethylketocyclazocine diminished the increase in heart rate elicited by post-ganglionic sympathetic nerve stimulation without interfering with the postsynaptic response to exogenous noradrenaline (Starke et al 1985). However, in that study the δ-receptor ligands DADLE and [Met]enkephalin and the μ-receptor agonist morphine, were reported to have no effect on the cardiac response to sympathetic stimulation. Likewise, in rat and guinea-pig isolated atria, responses to noradrenergic nerve stimulation and exogenous noradrenaline were shown to be unaffected by DADLE and [D-Ala^2, Met^5]enkephalinamide (Wong-Dusting & Rand 1985). Therefore, it has been proposed that only presynaptic opioid κ-receptors, and not μ- or δ-receptors, inhibit the release of noradrenaline from sympathetic neurons, at least in atria under in-vitro conditions. This contrasts with the present study, where opioid agonists acting on all three main opioid receptor types were effective in reducing the cardio-acceleration to sympathetic nerve stimulation. However, it should be noted that in the case of DADLE and glyol, but not ICI 204448, higher doses than those needed to produce a haemodynamic effect in anaesthetized rats were required to reduce the effects of cardiac sympathetic nerve stimulation. This might suggest that DADLE and glyol are not acting on their specific opioid-receptor subtypes. We have not determined the specific receptors on which each of the agonists are acting under our experimental conditions. However, we have shown that in both anaesthetized and pithed rats the effects of the δ-preferring agonist DADLE, are blocked by M8008, a δ-receptor antagonist. These results suggest that mainly δ-opioid receptors are involved in the actions of DADLE both in anaesthetized and pithed rats. The glyol-induced negative chronotropic response was also slightly attenuated by M8008. This suggests that either δ-receptors may be partially involved in the fall in heart rate produced by glyol, or that in the dose of M8008 used, this antagonist may be blocking some μ-receptors as well as δ-receptors. Further studies with specific antagonists for μ- and

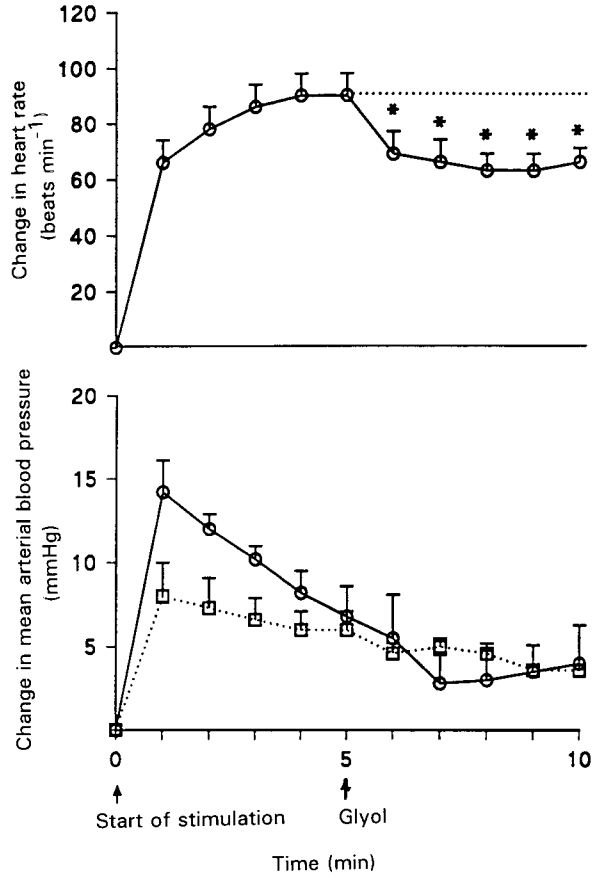

FIG. 4. The effects of glyol (2·5 mg kg^{-1}, n = 4), given intravenously, on the cardio-accelerator response and vasopressor responses to spinal cord (C7-T2) stimulation in pithed rats (○). The dotted line illustrates the maintained increase in heart rate as observed in the saline control group. Values are presented as mean ± s.e.m. Pre-stimulation values for heart rate and mean arterial blood pressure were 309 ± 10 beats min^{-1} and 57 ± 2 mmHg, respectively, in the glyol-treated group.

κ-receptors would need to be carried out in order to determine which particular receptor types underlie the effects of glyol and ICI 204448. Since the concentrations of DADLE and glyol required to modulate the effects of sympathetic nerve transmission were higher than those that caused haemodynamic effects in anaesthetized rats, it may be that for these agonists inhibition of noradrenaline release may account for only a part of their negative chronotropic effects.

DADLE was also shown to inhibit the pressor response to sympathetic nerve stimulation. In accord with this finding are studies where DADLE reduced the stimulation-induced vasoconstriction of the rabbit ear artery (Fukuda et al 1985), the rabbit mesenteric artery (Illes et al 1986) and [^{3}H]-noradrenaline overflow in the rabbit ileocolic artery (Von Kugelen et al 1985). These responses were sensitive to naloxone. Taken together, these results suggest that DADLE acts on opioid receptors located on the axon terminals of post-ganglionic sympathetic neurons innervating various blood vessels. Activation of these presynaptic receptors inhibits neuroeffector transmitter release and causes vasodilation. In the case of clonidine, a pressor response was observed on administration. This has been suggested to be due to a direct effect of clonidine on postjunctional α-receptors in vascular smooth muscle (Docherty & McGrath 1980).

The opioid-agonists attenuation of the cardio-acceleration response to sympathetic nerve stimulation was only observed with very high doses of the agonists. Such an effect would decrease, rather than increase, the severity of ventricular ectopic activity since catecholamines exacerbate such arrhythmias by enhancing automatic activity and by shortening the refractory period (Wit et al 1975). Furthermore, antiarrhythmic effects of various opioid agonists have now been reported (Sarne et al 1991; Pugsley et al 1992). These effects may result from a class I action. Therefore, in light of recent reports, it may be that the mechanism underlying the effects of opioid agonists on arrhythmias does not involve an interaction with opioid receptors but rather involves direct electrophysiological effects.

Acknowledgements

This work was supported by an SERC (CASE) award with Reckitt & Colman. We thank them for their financial support and Dr C. Smith of Reckitt & Colman, but currently with ICI plc, for his help and advice with the project.

References

Altura, B. T., Altura, B. M., Quirion, R. C. (1984) Identification of benzomorphan kappa-opiate receptors in cerebral arteries which subserve relaxation. Br. J. Pharmacol. 82: 459–466

Clo, C., Muscari, C., Pignatti, C., Bernardi, P., Ventura, C. (1985) Reduced mechanical activity of perfused rat heart following morphine or enkephalin peptides administration. Life Sci. 37: 1327–1333

Docherty, J. R., McGrath, J. C. (1980) The factors influencing the time course of drug action at α-adrenoceptors: an investigation of the effects of clonidine in the pithed rat. Br. J. Pharmacol. 68: 225–234

Fagbemi, O., Lepran, I., Parratt, J. R., Szekeres, L. (1982) Naloxone inhibits early arrhythmias resulting from acute coronary ligation. Br. J. Pharmacol. 76: 504–506

Fukuda, H., Hosoki, E., Ishida, Y., Moritoki, H. (1985) Opioid receptor types on adrenergic nerve terminals of rabbit ear artery. Br. J. Pharmacol. 86: 539–545

Gautret, B., Schmitt, H. (1984) Cardiac slowing induced by peripheral κ-opiate receptor stimulation in rats. Eur. J. Pharmacol. 102: 159–163

Gautret, B., Schmitt, H. (1985) Multiple sites for the cardiovascular actions of fentanyl in rats. J. Cardiovasc. Pharmacol. 7: 649–652

Gillan, M. G. C., Kosterlitz, H. W. (1982) Spectrum of the mu-, delta- and kappa-binding sites in homogenates of rat brain. Br. J. Pharmacol. 77: 461–468

Gillespie, J. S., McLaren, A., Pollock, D. (1970) A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. Br. J. Pharmacol. 40: 257–267

Handa, B., Lane, A. C., Lord, J. A. H., Morgan, B. A., Rance, M. J., Smith, C. F. C. (1981) Analogues of β-LPH 61–64 possessing selective agonist activity of μ-opiate receptors. Eur. J. Pharmacol. 70: 531–540

Illes, P., Pfeiffer, N., Limberger, N., Starke, K. (1983) Presynaptic opioid receptors in the rabbit ear artery. Life Sci. 33: 307–310

Illes, P., Ramme, D., Starke, K. (1986) Presynaptic opioid δ-receptors in the rabbit mesenteric artery. J. Physiol. 379: 217–228

Kosterlitz, H. W., Paterson, S. J. (1981) Tyr-D-Ala-Gly-MePhe-NH(CH$_2$)$_2$OH is a selective ligand for the μ-opiate binding site. Br. J. Pharmacol. 73: 299P

Lang, R. E., Hermann, K., Dietz, R., Gaida, W., Ganten, D., Kraft, K., Unger, T. H. (1983) Evidence for the presence of enkephalins in the heart. Life Sci. 32: 399–406

Lee, A. Y. S., Zhan, C. Y., Wong, T. M. (1984) Effects of β-endorphin on the contraction and electrical activity of the isolated perfused rat heart. Int. J. Peptide Protein Res. 24: 525–528

McIntosh, M., Kane, K. A., Parratt, J. (1991) The effects of opioid peptides on cardiovascular and sympathetic neurotransmission in rats. Br. J. Pharmacol. 104: 186P

McIntosh, M., Kane, K., Parratt, J. (1992) Effects of selective opioid receptor agonists and antagonists during myocardial ischaemia. Eur. J. Pharmacol. 210: 37–44

Parratt, J. R., Sitsapesan, R. (1986) Stereospecific antiarrhythmic effect of opioid receptor antagonists in myocardial ischaemia. Br. J. Pharmacol. 87: 621–622

Pugsley, M. K., Penz, W. P., Walker, M. J. A., Wong, T. M. (1992) Antiarrhythmic effects of U50,488H in rats subjected to coronary artery occlusion. Eur. J. Pharmacol. 212: 15–19

Romer, D., Buscher, H., Hill, R. C., Maurer, R., Petcher, T. J., Welle, H. B. A., Bakel, H. C., Akkerman C. K. (1980) Bremazocine; a potent long acting opiate κ-agonist. Life Sci. 27: 971–978

Sarne, Y., Flitstein, A., Oppenheimer, E. (1991) Antiarrhythmic activities of opioid agonists and antagonists and their stereoisomers. Br. J. Pharmacol. 102: 696–698

Schomig, A., Richardt, E. (1991) Cardiac sympathetic activity in myocardial ischaemia: release and effects of noradrenaline. In: Heusch, G., Ross, J. (ed.) Adrenergic mechanisms in myocardial ischaemia. Darmstadt: Steinkopf, pp 9–30

Shaw, J. S., Carroll, J. A., Alcock, P., Main, B. G. (1989) ICI 204448: a κ-opioid agonist with limited access to the CNS. Br. J. Pharmacol. 96: 986–992

Smith, C. F. C. (1987) Mecyprenorphine (RX8008M): a potent opioid antagonist with some δ selectivity. Life Sci. 40: 267–274

Starke, K., Schoffel, E., Illes, P. (1985) The sympathetic axons innervating the sinus node of the rabbit possess presynaptic opioid κ- but not μ- or δ-receptors. Naunyn Schmiedebergs Arch. Pharmacol. 329: 206–209

Sun, F.-Y., Zhang, A. Z. (1985) Dynorphin receptor in the blood vessel. Neuropeptides 5: 595–598

Vargish, T., Beamer, K. C., Daly, T., Head, R. (1987) Myocardial opiate receptor activity is stereospecific, independent of muscarinic receptor antagonism and may play a role in depressing cardiac function. Surgery 102: 171–177

Von Kugelen, I. V., Illes, P., Wolf, D., Starke, K. (1985) Presynaptic inhibitory opioid δ- and κ-receptors in a branch of the rabbit ileocolic artery. Eur. J. Pharmacol. 118: 97–105

Weihe, E., McKnight, A. T., Corbett, A. D., Hartschuh, W., Reinecke, M., Kosterlitz, H. W. (1983) Characterisation of opioid peptides in guinea-pig heart and skin. Life Sci. 33: 711–714

Weihe, E., McKnight, A. T., Corbett, A. D., Kosterlitz, H. W. (1985) Proenkephalin- and prodynorphin-derived opioid peptides in guinea-pig heart. Neuropeptides 5: 453–456

Wit, A. L., Hoffman, B. F., Rosen, M. R. (1975) Electrophysiology and pharmacology of cardiac arrhythmias. IX. Cardiac electrophysiologic effects of beta-adrenergic receptor stimulation and blockade. Am. Heart J. 90: 521–533

Wong, T. M., Lee, A. Y. S., Tai, K. K. (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. J. Mol. Cell. Cardiol. 22: 1167–1175

Wong-Dusting, H., Rand, M. J. (1985) Effect of [D-Ala^2,Met^5] enkephalinamide and [D-Ala^2, D-Leu^5] enkephalin on cholinergic and noradrenergic neurotransmission in isolated atria. Eur. J. Pharmacol. 111: 65–72

Zimmerman, D. M., Leander, J. D. (1990) Selective opioid receptor agonists and antagonists: research tools and potential therapeutic agents. J. Med. Chem. 33: 895–902