Br. J. Pharmac. (1983), **79**, 997–1002

Hypothalamic regulation of the cardiovascular and respiratory systems: role of specific opiate receptors

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1 Experiments were designed to evaluate the role of μ and δ opiate receptors in central cardiovascular control in the hypothalamic nucleus preopticus medialis of rats anaesthetized with pentobarbitone.

2 The highly selective μ opiate receptor agonist D-Ala^2-MePhe^4-Gly^5-ol-enkephalin was extremely potent in eliciting hypotension and bradypnoea; tachycardia was elicited by a low dose (0.064 nmol), but not by higher doses (0.64–6.4 nmol).

3 Other selective μ receptor agonists (morphine sulphate, morphiceptin) caused tachycardia at lower doses (0.64, 6.4 nmol), hypotension and bradypnoea after the highest dose (64 nmol).

4 The relatively selective δ receptor agonist D-Ala^2-D-Leu^5-enkephalin caused profound bradypnoea and hypotension at the high dose (64 nmol), tachycardia after the lowest dose (0.64 nmol), but bradycardia was found during the hypotension induced by the high dose (64 nmol).

5 All of the opiate/opioid effects were reversed by naloxone (0.5 mg kg^{-1}, i.v.).

6 It is concluded that μ receptors may mediate the cardiovascular and respiratory effects of opiates and opioid peptides in the nucleus preopticus medialis of the rat.

Introduction

There is accumulating evidence to support the hypothesis that endogenous opioids play a role in central cardiovascular and respiratory regulation. First, opioid peptides and opiate receptors are found in brain nuclei which are known to control haemodynamic and respiratory functions (Simantov, Kuhar, Uhl & Snyder, 1977; Dupont, Lepine, Langelier, Merand, Rouleau, Vaudry, Gros & Barden, 1980). Second, central opioid systems have been implicated in the pathophysiology of shock due to various causes (Faden & Holaday, 1980; Holaday & Faden, 1980; Janssen & Lutherer, 1980). Third, opioid peptides appear to play a role in the mediation of hypotensive responses to centrally acting antihypertensive drugs (Farsang & Kunos, 1979). Finally, potent cardiovascular effects are elicited by opioid peptides following administration into the lateral ventricle (i.c.v.) (Yukimura, Stock, Stumpf, Unger & Ganten, 1981; Feldberg & Wei, 1981) or the cisterna magna (Bolme, Fuxe, Agnati, Bradley & Smythies, 1978).

These latter studies have been used to suggest specific cardioregulatory roles for various endogenous opioids. Yet, the cardiovascular responses to cerebroventricular administration of opioids may reveal little about the discrete functions of the opiates in specific cardiovascular nuclei since cerebroventricular injections spread to many nuclei and pathways in the brain, and the effects recorded probably reflect a summation of differential actions in various brain regions. This possibility is consistent with the frequently biphasic responses observed after i.c.v. or cisterna magna administration of opiates (Laubie, Schmitt, Vincent & Remond, 1977; Bolme *et al.*, 1978). Moreover, recent studies from our laboratory indicate that the injection site is a major factor determining the cardiovascular response to centrally administered opiates (Feuerstein & Faden, 1982; Hassen, Feuerstein & Faden, 1982a,b).

The identification of multiple opiate receptors in the brain (Lord, Waterfield, Hughes & Kosterlitz, 1977), and the heterogeneous distribution of different opiate receptor types in various brain nuclei (Goodman, Snyder, Kuhar & Young, 1980; Pfeiffer, Pasi, Mehraein & Herz, 1982), raise the possibility that the cardiovascular and respiratory effects of centrally administered opioids are receptor-specific as well as site-specific.

Unfortunately, the opioid peptides used in previous studies (i.e., Leu^5-enkephalin, D-Ala^2-Met^5-enkephalinamide) have significant affinities for more than one class of receptors (i.e., μ and δ). Thus, such studies cannot be used to distinguish receptor specific cardiovascular activity.

However, development of newer opiate agonists with more selective receptor affinities provides the opportunity to examine the relationship between opiate receptor type and cardiovascular function. The following studies were aimed at addressing this issue by microinjecting selective opiate receptor ligands into a discrete hypothalamic region (nucleus preopticus medialis; POM) known to play an important role in cardiovascular control (Brody, Haywood & Tuow, 1980), and in which the presence of opioid peptides and receptors have already been established (Goodman *et al.*, 1980; Law, Loh & Li, 1979).

Methods

Male Sprague-Dawley rats (Taconic Farms) weighing 280–300 g were anaesthetized with pentobarbitone (50 mg kg^{-1}, i.p.) and the left femoral artery and vein cannulated (PE 50). Rats were placed on a stereotaxic device (DKI, CA) and a hole drilled through the cranium according to coordinates measured from the Bregma: AP = 0.1 mm, L = 0.6 mm for injections into the POM. A pulled glass capillary (o.d. 70–80 μm) was inserted to a depth of 7.8 mm from the surface of the skull and the rats were allowed to stabilize over 20–30 min with no additional anaesthetics. Rectal temperature was maintained at 37.7–38°C with a controlled heating pad (YSI, model 73A). The arterial line was attached to a Narco microtransducer (RP 1500i) for blood pressure and heart rate recording; respiratory rate and relative pneumatic impedance (RPI) were recorded by subcutaneous electrodes through a Narco RPI coupler (type 7212). Automatic sampling (every minute) of blood pressure, heart rate and respiratory rate was done by a computerized Narco 80 DAS dynograph. Opiates or vehicle (0.9% saline, sterile, pyrogen-free) were injected in 1 μl over 30 s by a 10 μl Hamilton syringe attached to the glass capillary through a PE 20 catheter. Each rat received only one injection of the drug or saline followed by one injection of naloxone. Naloxone (Endo Laboratories) was injected at the peak of the response (0.5 mg kg^{-1}, i.v.), as determined by preliminary experiments, and the recording continued for 15 min. Rats were subsequently killed, and the brain removed and frozen on dry ice for serial sectioning. Thionine (0.1%) stained sections (50 μm) were examined microscopically and the tip of the injection cannula was found in the region of the POM, A7020-A6360, according to König & Klippel (1967).

The following drugs were used: morphine sulphate (Mallinkrodt) μ prototype; D-Ala^2-D-Leu^5-enkephalin (DADL, Peninsula, mol.wt. 569), a relatively selective δ receptor agonist (Kosterlitz, Lord, Paterson & Waterfield, 1980; Kosterlitz, Paterson & Robson, 1981); D-Ala^2-MePhe^4-Gly^5-ol-enkephalin (DAGO, kindly provided by Dr Maurer, Sandoz, Basel Switzerland, mol.wt. 513), a selective μ recep-

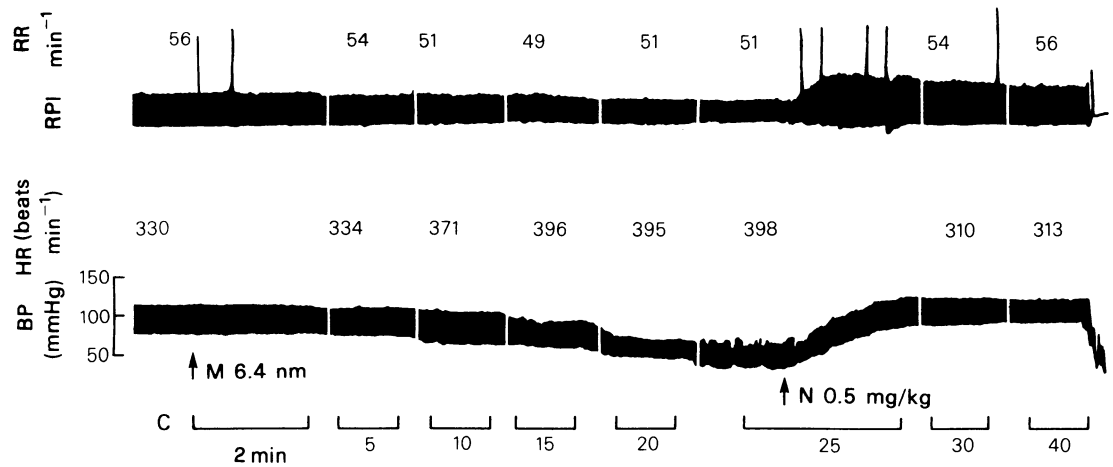

Figure 1 Cardiovascular and respiratory effect of morphine, microinjected into the nucleus preopticus medialis (POM) of rats anaesthetized with pentobarbitone. This recording is typical of experiments in which morphine (M), 6.4 nmol was given as denoted: M after a control period (C). The upper panel is the recording of the relative pneumatic impedance (RPI) and respiration rate (RR min^{-1}). Naloxone (N) was injected at N. This recording demonstrates the suppression of both volume and rate of respiration by the opiate and opioid peptides used in this study.

tor agonist (Handa, Lane, Lord, Morgan, Ranu & Smith, 1981; Kosterlitz & Paterson, 1981), and morphiceptin (MPCT, Peninsula, mol.wt. 522), a selective μ receptor agonist (Chang, Killian, Hazum & Cuatrecasas, 1981).

The dose of naloxone used in this study ($0.5\,mg\,kg^{-1}$, i.v.) was selected to antagonize the opiate effects since, in preliminary studies, we have observed significant cardiac depression by higher doses ($2\,mg\,kg^{-1}$) injected to pentobarbitone-anaesthetized rats.

Analysis of variance and Duncan's Multiple Range Test (Bruning & Kintz, 1979) were used for statistical evaluation of the changes elicited by the drugs and the paired Student's *t* test was used to evaluate naloxone effects. Data in text and figures are mean ± s.e.mean for the indicated number of rats in each group.

Results

The mean arterial blood pressure (MBP) before drug administration was 90.2 ± 3.1 mmHg; heart rate was 344 ± 12 beats min^{-1} and respiratory rate was 72 ± 3 breaths min^{-1}. No differences were found in the control values among the various experimental groups. A typical experiment of morphine injection is shown in Figure 1.

Effects of morphine, DAGO, DADL and MPCT on blood pressure

All of the opiates tested in this study affected MBP (Figure 2a). However, large quantitative (and some qualitative) differences were noted. DAGO caused profound hypotension at a subnanomolar dose (0.64), whereas no significant changes in MBP were demonstrated by any of the other opiates at this dose. Morphine caused slight elevation of MBP + 15 ± 6 mmHg ($n = 6$, $P < 0.05$) at 6.4 nmol, while no significant effects were observed with DADL and MPCT. Only the highest dose of DADL, morphine and MPCT (64 nmol) induced a significant depressor effect: DADL −69 ± 5 mmHg ($n = 9$); morphine −52 ± 9 mmHg ($n = 5$); MPCT −21 ± 5 mmHg ($n = 7$, $P < 0.001$).

Effects of morphine, DAGO, DADL and MPCT on heart rate

All of the opiates injected into the POM caused cardiac acceleration (Figure 2c). Significant elevation of heart rate up to 40 ± 6 beats min^{-1} ($n = 9$, $P < 0.01$) by DAGO was observed at a dose (0.064 nmol) that had no significant effect on MBP or respiratory rate. Tachycardia, without a significant change in MBP or respiration, was also elicited by

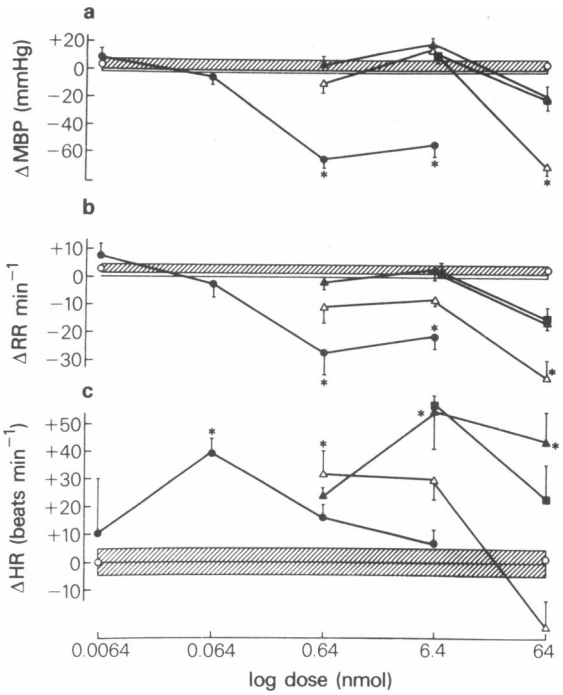

Figure 2 Cardiovascular and respiratory effects of morphine (▲), D-Ala² - MePhe⁴-Gly⁵ - ol-enkephalin (●, DAGO), morphiceptin (■, MPCT) and D - Ala²-D-Leu⁵-enkephalin (△, DADL) microinjected into the nucleus preopticus medialis (POM) of pentobarbitone-anaesthetized rats: (a) maximal change in mean blood pressure (ΔMBP); (b) maximal change in respiratory rate (ΔRR min^{-1}); and (c) maximal change in heart rate (ΔHR in beats min^{-1}). (○) = Saline. Data are mean of 5–9 rats; vertical lines show s.e.mean. Abscissa scale denotes the dose in nanomole. Asterisks denote statistical significance by the Duncan's Multiple Range Test ($P < 0.01$) except for the heart rate effect of 0.064 nmol DAGO which was compared to saline injected group by Student's *t* test ($P < 0.001$).

morphine, DADL and MPCT (Figure 2c). However, higher doses of the opiates showed less of a cardiac accelerating effect. This phenomenon was seen especially in the DAGO and MCPT experiments; the highest dose of DADL tended to cause bradycardia in spite of the severe hypotension.

Effects of morphine, DAGO, MPCT and DADL on respiratory rate

All of the opiates used in this study depressed respiration (Figure 2b). DAGO was the most potent agent in this regard, and profound respiratory depression (−28 ± 7 breaths/min, $n = 5$) was induced by the subnanomolar dose (0.64). DADL also lowered respiratory rate, but significant effects were seen only

at higher doses: -9 ± 2 breaths/min, ($n=7$, $P<0.01$) at 6.4 nmol and -37 ± 7 breaths/min, ($n=6$, $P<0.001$) at 64 nmol. Neither morphine nor MPCT affected respiratory rate at doses below 6.4 nmol, but respiratory depression was observed after a 64 nmol dose of morphine -22 ± 7 breaths min^{-1}, $P<0.01$) or MPCT (-15 ± 4 breaths min^{-1}, $P<0.01$) (Figure 2b). However, even at this high dose the magnitude of the respiratory effect of morphine and MPCT was significantly less than the respiratory effect of DAGO at a dose two orders of magnitude smaller.

Effects of naloxone on the cardiovascular effects of morphine, DAGO, MPCT and DADL

Injection of naloxone at the peak of the opiate effects reversed the blood pressure, heart rate and respiratory effects of all the opiates. The changes observed after naloxone administration were similar in magnitude to the changes induced by the opiates (Figure 3 a–c). Naloxone also elicited significant cardiovascular/respiratory changes in experiments where the opiate had no apparent effect by itself. For example, 0.064 nmol DAGO had no significant effect on MBP or respiratory rate; however, naloxone administration resulted in a significant increase in MBP ($+14 \pm 6$ mmHg, $P<0.05$) and an even more pronounced increase in respiratory rate $+15 \pm 4$ breaths min^{-1}, ($n=8$, $P<0.01$). This phenomenon was also observed with morphine (0.64 nmol); rats which had no change in MBP or respiratory rate after the opiate showed significant tachypnoea after naloxone administration (Figure 3b).

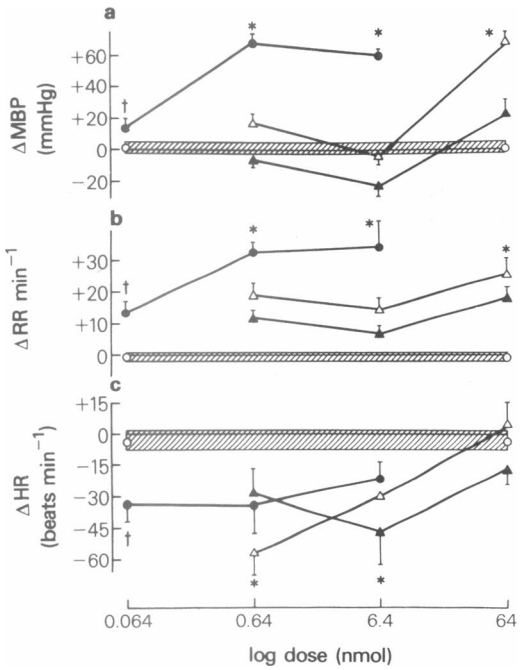

Figure 3 Effect of naloxone (0.5 mg/kg, i.v.) on blood pressure, heart rate and respiration of opiate-treated rats. (a) maximal change of blood pressure (ΔMBP); (b) maximal change in respiration rate (ΔRR min^{-1}); and (c) maximal change in heart rate (ΔHR in beats min^{-1}). The symbols of the various opiates are as in Figure 2. Data are mean of 5–9 rats; vertical lines show s.e.mean. Abscissa scale denotes the dose in nanomol. Asterisks denote statistical significance by the Duncan's Multiple Range Test: *$P<0.01$; †denotes statistical significance between DAGO and saline as follows (a) †$P<0.05$; (b) †$P<0.01$; and (c) † $P<0.001$.

Discussion

The data presented in this paper indicate that the POM, which comprises a major part of the anteroventral hypothalamus of the rat, is a sensitive site for cardiovascular modulation by morphine and opioid peptides. All the opiates used in this study produced changes in blood pressure, heart rate or respiration when administered into the POM; however, large quantitative differences were found among the various opiate agonists. The most active opiate was DAGO, which caused a significant increase in heart rate at a dose of 0.064 nmol. Cardiac acceleration was also elicited by POM injections of morphine, MPCT and DADL at higher doses. At higher doses, each of the opiates used in this study caused hypotension, although DAGO again was most potent. DAGO had the highest affinity for the μ receptor of the various μ agonists tested; it also has a 200 to 400 fold selectivity for μ over δ receptors in binding assays (Handa *et al.*, 1981; Kosterlitz *et al.*, 1981). Although DADL has some selectivity for δ receptors, it also possesses substantial affinity for the μ receptor. Thus, the cardioacceleratory and vasodepressor effects of this opiate agonist, which are similar to those of DAGO but require far higher doses, probably result from actions at the μ receptor. Taken together, these findings are consistent with the conclusion that μ receptors mediate the cardioaccelerator and vasodepressor actions of opioids in this region.

This conclusion contrasts with recent suggestions that the cardiovascular effects of opioid peptides in the forebrain are mediated by δ opiate receptors (Ganten, Unger, Scholkens, Rascher, Speck & Stock, 1981). However, previous studies utilized opiate peptides which have low specificity for μ or δ receptors (i.e., Leu^5-enkephalin D-Ala^2-Met^5-enkephalin); moreover, much higher doses of these peptides injected i.c.v. were needed to elicit tachycardia, as compared with the present study. These differences between i.c.v. and parenchymal

microinjection studies underscore the limitations of i.c.v. injection studies in determining the autonomic effects of opiates. Such injections produce effects which may be due to stimulation of various brain areas which may exhibit different or opposing cardiovascular activities (Feuerstein & Faden, 1982).

The suppression of the respiratory rate elicited by microinjections of the opiates into the POM indicates that this hypothalamic region might also be an important site for respiratory control by the endogenous opioid system. It appears that μ receptors may also mediate this effect since the order of potency for respiratory suppression was DAGO > DADL > morphine/MPCT. The respiratory effects of the opiates in the POM are consistent with previous studies in which various opiates and opioid peptides were administered systemically or i.c.v (Sisten, Van Ree & De Jong, 1982; Zobrist Allerton & Isom, 1982; Hurle Mediavilla & Fiorez, 1982; McGillard & Takemori, 1978). However, Hurle *et al.* (1982) indicated that brainstem nuclei mediate the opiate respiratory depression; this study provides evidence that forebrain nuclei as well may be involved in respiratory control by opiates and further supports recent studies indicating that the anteroventral hypothalamus is an important site for respiratory regulation by other neuropeptides (Farber, Connors, Gisolfi, McCaffree & Smith, 1981).

The profound depression in respiratory rate observed after injection of the higher doses of DAGO and DADL into POM might also contribute to the cardiovascular depression observed at these doses. This possibility is supported by recent observations that depressor responses elicited by D-Ala^2-Met^5-enkephalin injection into hindbrain nuclei of pentobarbitone-anaesthetized rats are abolished by artificial respiration (Elghozi, Bellet & Meyer, 1981).

It is noteworthy that heart rate was not elevated during the profound hypotension induced by DAGO and DADL. Since reflex tachycardia is anticipated during severe hypotension, the lack of tachycardia (and even relative bradycardia in DADL-treated rats) raises the possibility of a concomitant activation of vagal tone or disruption of the baroreflex mechanism. There is some support for the latter possibility in recent studies showing reduced sensitivity of the baroreflex mechanism upon microinjections of μ-receptor agonists into the cisterna magna (Petty & Reid, 1982).

Naloxone administration rapidly and completely reversed all the cardiovascular and respiratory effects of the opioids. Since the dose of naloxone used in this study was relatively high, this finding does not help to distinguish the specific opiate receptors involved. However, it is noteworthy that naloxone administration after the lowest dose of DAGO or morphine caused significant changes in MBP and respiration, even though no significant changes in these parameters were observed prior to naloxone administration. This phenomenon may indicate that compensatory mechanisms effectively counterbalance changes induced by the opioids at low doses, and rebound changes are therefore seen when the opiate effect is rapidly antagonized. Alternatively, such a finding may reflect simultaneous activation by opioids of two classes of opiate receptors (which have opposing cardiovascular actions), only one of which is naloxone-sensitive.

In summary, the present findings are consistent with μ receptors mediating cardiovascular/respiratory effects of opioid peptides in a discrete forebrain nucleus of the hypothalamus. However, we cannot entirely exclude the possibility that δ receptors also mediate the cardiovascular responses elicited by the opiate/opioid peptides in this region. In addition, this study stresses the importance of discrete parenchymal administration of selective receptor ligands (as opposed to ventricular administration) in investigating the central autonomic actions of opioids. Finally, the present experiments indicate that the POM, which comprises a major part of the anteroventral hypothalamic region, may be a central site for cardiovascular and respiratory control by the endogenous opioid system.

This work was supported by the Uniformed Services University of the Health Sciences Protocol No. RO9211. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. The experiments reported herein were conducted according to the principals set forth in the *Guide for the Care and Use of Laboratory Animals,* Institute of Laboratory Animal Resources, National Research Council DHEW Pub. (NIH) 74-23. The authors wish to thank Mrs Mary Spies for her excellent technical assistance, and Miss Eleanor M. Bell for her preparation of this paper.

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(Received March 9, 1983
Revised April 12, 1983.)