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Investigation of the central sites at which morphine acts to cause hypertension in conscious rabbits

[1]C.N. May, C.J. Whitehead, *M.R. Dashwood & C.J. Mathias

Department of Medicine, St. Mary's Hospital Medical School, London, W2 1NY and *Department of Physiology, Royal Free Hospital School of Medicine, London, NW3

1 In conscious rabbits intracerebroventricular (i.c.v.) morphine (10 and 50 $\mu g\,kg^{-1}$) caused a dose-related increase in plasma noradrenaline and adrenaline, respiratory depression and sedation. The increase in sympatho-adrenal outflow resulted in hypertension accompanied by bradycardia and the increase in adrenaline secretion caused hyperglycaemia. Morphine (1 $\mu g\,kg^{-1}$ i.c.v.) and i.c.v. saline had no effect.

2 The same doses of morphine given intracisternally (i.c.) caused bradycardia and a similar degree of respiratory depression to i.c.v. morphine, but no significant increase in blood pressure and only a small, gradual rise in plasma adrenaline.

3 Intravenous naloxone (1 $mg\,kg^{-1}$) did not block the hypertension, hyperglycaemia or increase in plasma catecholamines that followed i.c.v. morphine, but prevented the respiratory depression and sedation.

4 Ganglionic blockade with pentolinium prevented the rise in plasma catecholamines, blood pressure and plasma glucose induced by i.c.v. morphine.

5 These findings demonstrate that the increased sympathoadrenal outflow following i.c.v. morphine results from an action on periventricular structures. The resultant increase in plasma catecholamines, which is largely naloxone resistant, accounts for the hypertension and hyperglycaemia. The bradycardia is probably partly baroflex mediated and partly due to an increase in vagal tone as a result of stimulation of brainstem opioid receptors. The respiratory depression is probably due to an action of morphine on brainstem opioid receptors.

Introduction

Morphine given intravenously caused a centrally mediated increase in sympatho-adrenal outflow in conscious, but not anaesthetised rabbits (May *et al.*, 1988; 1989). The increased sympathetic nerve activity and increased secretion of adrenaline caused a sustained increase in blood pressure (BP) accompanied by a fall in heart rate (HR). The increased adrenaline secretion accounted for the hyperglycaemia. These effects together with the respiratory depression and sedation were prevented by intravenous (i.v.) or intracerebroventricular (i.c.v.) naloxone indicating dependence on stimulation of central opioid receptors (May *et al.*, 1988; 1989).

We have investigated the central sites at which morphine acts by comparing the responses to i.c.v. and intracisternal (i.c.) administration of morphine in conscious rabbits. The effects of a range of doses given by these two routes on cardiovascular and respiratory function and on sympatho-adrenal outflow have been compared.

[1] Author for correspondence at present address: The Howard Florey Institute, Department of Experimental Physiology and Medicine, University of Melbourne, Parkville 3052, Victoria, Australia.

Methods

Surgical procedures

Two groups of male Sandy Half-lop rabbits (National Institute for Medical Research, Mill Hill) allowed free access to food (RHM R14, Labsure Animal Diets) and water and weighing between 2.4 and 3.8 kg were studied.

For injection into the right lateral ventricle, cannulae (Harvard Apparatus) were implanted aseptically under general anaesthesia (Saffan, Glaxovet). Coordinates were 1.0 mm caudal to bregma, 3.0 mm

lateral to midline and 8.5 mm below the dura. Cannulae were screwed into a hole drilled in the skull and fixed with dental cement which also enveloped 3 stainless steel screws screwed into the skull. Drugs were injected i.c.v. by a Hamilton syringe with a 25 g needle that protruded 1 mm from the cannula tip.

For injection into the cisterna magna a modification of the catheter described by Head *et al.* (1983) was used. Catheters were made from PP10 polythene tubing (i.d. 0.28 mm, o.d. 0.61 mm, Portex) one end of which was warmed and stretched to give an internal diameter of approximately 0.1 mm. A 4 cm length of silicone tubing (0.5 i.d., 1.0 mm o.d.) was expanded by soaking in diethyl ether which enabled it to be sleeved over the PP10 tube leaving 0.5 cm of the thinned tip protruding. The thinned end was bent back on to the silicone tube, held with tape and heated to 60°C after which it remained set in position. Animals were anaesthetised with Saffan, the atlanto-occipital membrane exposed and the tip of the cannula was inserted through a hole made with a 25 g needle. The catheter was held in place by a suture (6-0) which was passed through the silicone tube and membrane. Two further sutures were inserted through the periosteum overlying the occipital protruberance and tied around the silicone tube. The proximal end of the silicone tube was sutured to the muscle under the skin, and 4 cm of the PP10 tubing was left to protrude from the skin at the back of the neck. This was plugged and could be repeatedly used. The dead volume of the catheter was 10 μl. These catheters have remained patent and given reproducible responses for up to 6 months. Drugs were administered from a Hamilton syringe with a 30 g needle.

The injection site was confirmed at the end of a series of experiments by injection of 100 μl of bromophenol blue (1%), followed by 50 μl of saline i.c.v. or 20 μl i.c. The animal was killed by an overdose of pentobarbitone, the brain removed and the distribution of dye examined.

Animals were not used for at least 7 days post-operation. Before each experiment cannulae were inserted into the central artery and marginal vein of the ear under 1% lignocaine local anaesthesia. The arterial cannula was connected to a pressure transducer (Bell and Howell) and BP and HR were recorded on a Devices polygraph. During the experiment the rabbits sat on a grid (30 cm long) with solid plastic sides (14 cm high) adjusted to suit the width of the rabbit. Intervals of at least 7 days were allowed between studies.

Blood collection and analysis

Plasma from blood (2 ml) collected from the arterial cannula into fluoride oxalate tubes was used for the measurement of plasma glucose by a glucose oxidase method using a Chem Lab analyser. Catecholamines were measured in plasma from blood (2.5 ml) collected into cooled tubes containing 50 μl of EGTA (0.095%)/glutathione (0.06%) and kept on ice until centrifugation. Catecholamines were separated by h.p.l.c. and detected by electrochemical detection (May *et al.*, 1988). Arterial blood (100 μl) was collected into a capillary tube for the measurement of blood gases (Corning 158 pH/blood gas analyser).

Drugs

Drugs were dissolved in sterile, non-pyrogenic saline and doses refer to the salts. For i.c.v. and i.c. administration morphine sulphate (Macarthys) was given in 100 μl injected over 1 min, followed by 50 μl of saline (i.c.v.) or 20 μl of saline (i.c.). The treatments for the dose-response study were given in a randomised order. Naloxone hydrochloride (Sigma) was given either i.v. in 1 ml (0.5 or 1.0 mg kg^{-1}) via the marginal ear vein or i.c.v. in 100 μl (50 or 100 μg kg^{-1}) 15 min before morphine. Pentolinium (May and Baker) was given i.v. as a bolus dose (5 mg kg^{-1} in 1 ml kg^{-1}) 10 min before i.c.v. morphine.

Data analysis

Data are presented as means $\pm$ s.e.mean. Comparisons between treatment groups were made on differences from baseline values by use of the SAS statistical programme. The baseline values for mean arterial pressure (MAP) and HR consisted of the mean of the six readings taken during the 30 min control period. A logarithmic transformation was performed on the values for glucose, noradrenaline and adrenaline before analysis. The data were subjected to analysis of variance and where the null hypothesis was rejected Scheffe's comparisons were performed on the means; $P < 0.05$ was considered significant.

Results

Cardiovascular effects of i.c.v. and i.c. morphine

In conscious rabbits ($n = 5$) i.c.v. morphine produced a dose-related increase in MAP accompanied by a fall in HR. After i.c.v. morphine (50 μg kg^{-1}) the maximum increase in MAP of 43 ± 4 mmHg ($P < 0.05$) occurred after 15 min and MAP remained significantly elevated above baseline values for the 120 min experimental period. After i.c.v. morphine HR fell by 46 ± 17 beats min^{-1} ($P < 0.05$) after 10 min and by 70 ± 11 beats min^{-1} ($P < 0.05$) after

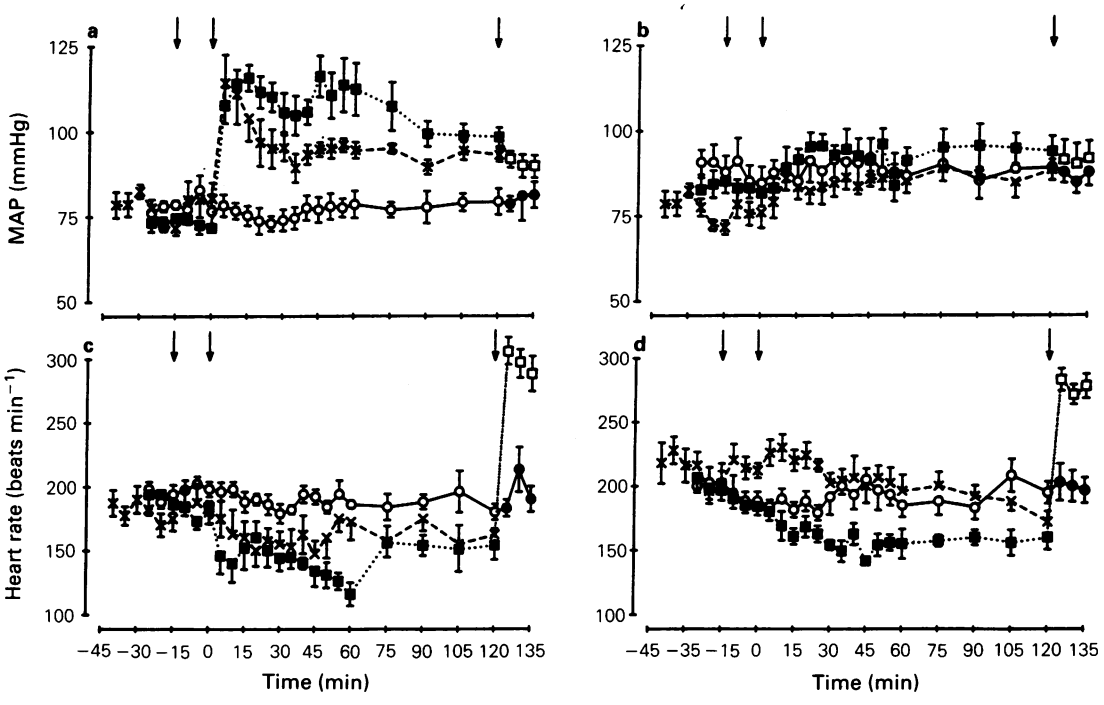

Figure 1 Cardiovascular responses to morphine or saline given either intracerebroventricularly (i.c.v.) (a, c) or intracisternally (i.c.) (b, d) in conscious rabbits. The effect on (a, b) mean arterial pressure (MAP) and (c, d) heart rate of saline (100 μl) (○——○) or morphine (50 $\mu g\,kg^{-1}$) (■·····■) given at 0 min either i.c.v. ($n = 5$) or i.c. ($n = 6$) followed by i.v. naloxone (0.5 $mg\,kg^{-1}$) given 120 min after saline (●——●) or morphine (□·····□), and the effect of i.v. naloxone (1 $mg\,kg^{-1}$) given at −15 min on the response to i.c.v. ($n = 4$) and i.c. ($n = 5$) morphine (50 $\mu g\,kg^{-1}$) (×– – –×). Each point represents the mean and vertical lines show s.e.mean.

60 min (Figure 1). After i.c.v. morphine (10 $\mu g\,kg^{-1}$) the changes in MAP and HR followed a similar time course. MAP increased by 21 ± 5 mmHg ($P < 0.05$) and HR fell by 31 ± 7 beats min^{-1} after 10 min; these changes in MAP were significantly greater than after i.c.v. saline but significantly less than after i.c.v. morphine (50 $\mu g\,kg^{-1}$). After 60 min MAP was elevated by 25 ± 8 mmHg ($P < 0.05$) and HR reduced by 43 ± 6 beats min^{-1}, and after 120 min MAP was elevated by 11 ± 5 mmHg and HR reduced by 19 ± 13 beats min^{-1}. There were no cardiovascular changes following i.c.v. morphine (1 $\mu g\,kg^{-1}$) or i.c.v. saline (100 μl).

Naloxone (0.5 $mg\,kg^{-1}$, i.v.) given 120 min after the 50 $\mu g\,kg^{-1}$ dose of i.c.v. morphine reduced MAP, although not to baseline levels, and caused an immediate increase in HR to 120 ± 12 beats min^{-1} above baseline ($P < 0.05$) (Figure 1). There was a similar rise in HR of 115 ± 10 beats min^{-1} when naloxone (0.5 $mg\,kg^{-1}$) was given 120 min after the 10 $\mu g\,kg^{-1}$ dose of morphine ($P < 0.05$), but an increase of only 10 ± 2 beats min^{-1} when it was given 120 min after the 1 $\mu g\,kg^{-1}$ dose ($P < 0.05$). After pretreatment with naloxone (1 $mg\,kg^{-1}$, i.v.), i.c.v. morphine (50 $\mu g\,kg^{-1}$) caused a similar initial increase in MAP which was followed by a more rapid fall than after morphine alone; the fall in HR was not affected by naloxone except at 55 and 60 min after morphine when HR in the naloxone-treated group was significantly greater (Figure 1). We have previously shown that, in conscious rabbits, i.v. naloxone (1 $mg\,kg^{-1}$) does not alter blood pressure, HR or plasma catecholamine levels (May *et al.*, 1988). In additional studies pretreatment with i.c.v. naloxone (100 $\mu g\,kg^{-1}$), given 15 min before i.c.v. morphine (50 $\mu g\,kg^{-1}$), or 50 $\mu g\,kg^{-1}$ naloxone given 15 min before 10 $\mu g\,kg^{-1}$ morphine, did not prevent the morphine-induced hypertension and bradycardia.

The same doses of morphine given i.c. resulted in significantly smaller changes in MAP than after i.c.v. administration. In six conscious rabbits i.c. morphine (50 $\mu g\,kg^{-1}$) caused a gradual increase in MAP and fall in HR; over 30 min MAP increased by 9 ± 4 mmHg and HR fell by 39 ± 6 beats min^{-1} but these changes were not significant (Figure 1). After i.c. morphine (10 $\mu g\,kg^{-1}$) HR fell by 20 ± 9

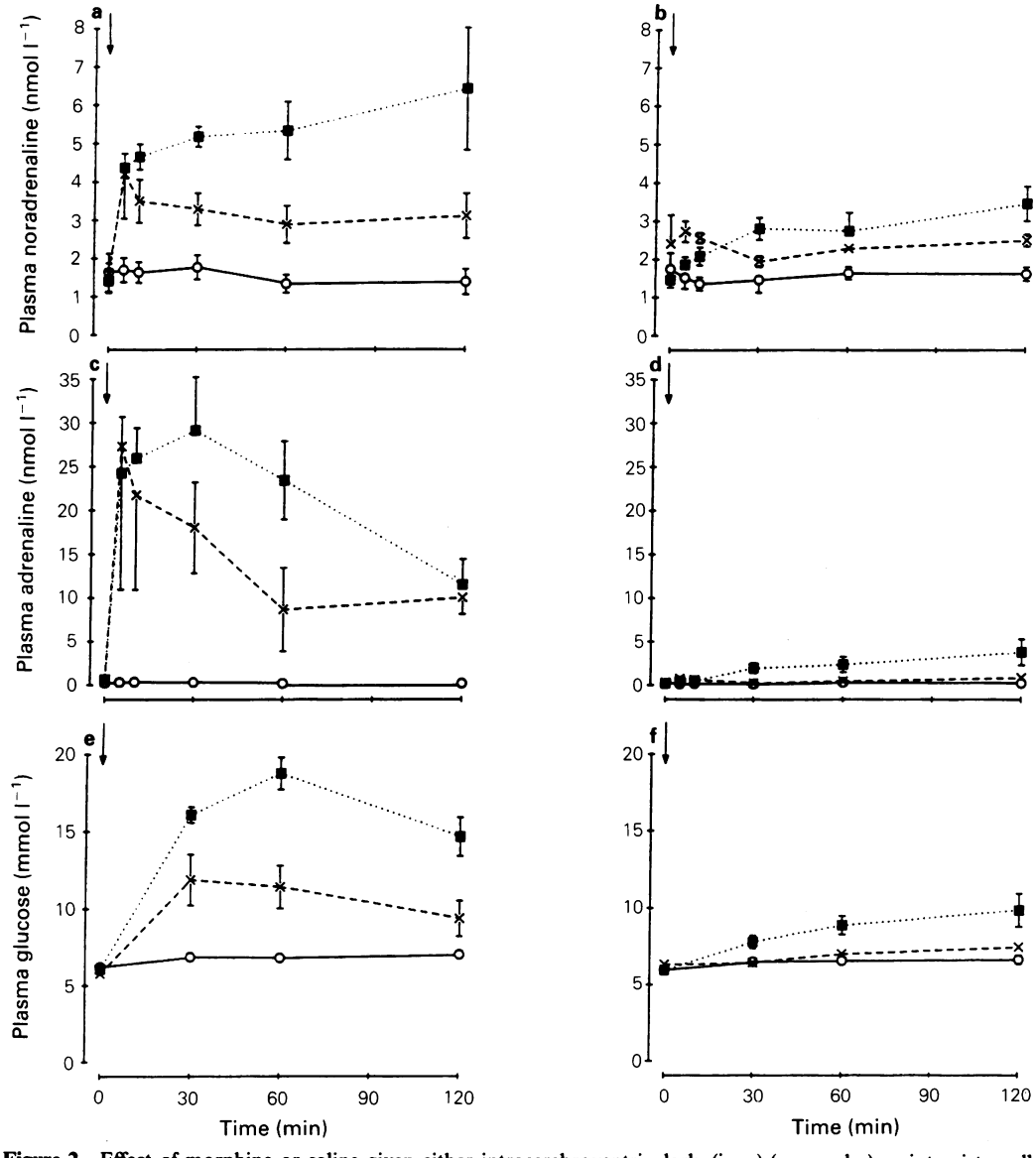

Figure 2 Effect of morphine or saline given either intracerebroventricularly (i.c.v.) (a, c and e) or intracisternally (i.c.) (b, d and f) on plasma levels of (a, b) noradrenaline, (c, d) adrenaline and (e, f) glucose in conscious rabbits. The effect of saline (100 μl) (○——○) or morphine (50 μg kg^{-1}) (■·····■) given at 0 min either i.c.v. ($n = 5$) or i.c. ($n = 6$) followed by i.v. naloxone (0.5 mg kg^{-1}) given 120 min after saline (●——●) or morphine (□·····□), and the effect of i.v. naloxone (1 mg kg^{-1}) given at −15 min on the response to i.c.v. ($n = 4$) and i.c. ($n = 5$) morphine (50 μg kg^{-1}) (×– – –×). The control samples were taken before the administration of drugs or vehicle. Each point represents the mean and vertical lines indicate s.e.mean.

beats min^{-1} over 30 min and this was accompanied by a rise in MAP of 5 ± 30 mmHg. There were no changes after i.c. morphine (1 μg kg^{-1}) or i.c. saline (100 μl).

Naloxone (0.5 mg kg^{-1}, i.v.) given 120 min after i.c. morphine (50 μg kg^{-1}) had no effect on MAP but increased HR by 90 ± 8 beats min^{-1} ($P < 0.05$) (Figure 1). HR increased by 62 ± 10 beats min^{-1}

($P < 0.05$) when naloxone (0.5 mg kg^{-1} i.v.) was given 120 min after i.c.v. morphine (10 μg kg^{-1}), but there was no effect when it was given after i.c. morphine (1 μg kg^{-1}) or i.c. saline. Pretreatment with naloxone (1 mg kg^{-1}, i.v.) reduced the BP response to i.c. morphine (50 μg kg^{-1}) and prevented the fall in HR (Figure 1).

Effects of i.c.v. and i.c. morphine on plasma catecholamines and plasma glucose

After i.c.v. morphine (50 μg kg^{-1}), plasma noradrenaline increased by 3.0 ± 0.5 nmol l^{-1} ($P < 0.05$) within 5 min and rose continually throughout the remaining 120 min to reach 5.1 ± 0.8 nmol l^{-1} above control ($P < 0.05$) (Figure 2). Plasma adrenaline reached a peak of 28.5 ± 6.3 nmol l^{-1} above control ($P < 0.05$) after 30 min and then fell, but remained significantly above control values after 120 min. After i.c.v. morphine (10 μg kg^{-1}) the initial rise in noradrenaline (2.0 ± 0.4 nmol l^{-1}) after 5 min was similar to that after the higher dose but the increases were less after 30 min (2.6 ± 0.1 nmol l^{-1}), 60 min (4.0 ± 0.5 nmol l^{-1}) and 120 min (3.0 ± 0.5 nmol l^{-1}). The increases in plasma adrenaline after 5 min (19.9 ± 6.4 nmol l^{-1}), 30 min (24.1 ± 5.7 nmol l^{-1}), 60 min (25.4 ± 5.7 nmol l^{-1}) and 120 min (12.2 ± 2.6 nmol l^{-1}) were similar to those after 50 μg kg^{-1} of morphine. There was a small transient rise in both catecholamines 5 min after 1 μg kg^{-1} morphine i.c.v. and no effect after i.c.v. saline. Pretreatment with naloxone (1 mg kg^{-1}, i.v.) did not alter the initial increase in plasma noradrenaline and adrenaline after i.c.v. morphine (50 μg kg^{-1}), but thereafter the levels of both catecholamines fell more rapidly than in the absence of naloxone (Figure 2).

After i.c. morphine (50 μg kg^{-1}) there was a small gradual increase in plasma adrenaline which was significant from 10 min after morphine, but there were no significant changes in plasma noradrenaline. The changes in adrenaline were reduced by pretreatment with naloxone (1 mg kg^{-1}, i.v.) (Figure 2). There was no change in catecholamines after the lower doses of i.c. morphine (1 and 10 μg kg^{-1}).

There was a dose-related hyperglycaemia after i.c.v. morphine (Figure 2). The maximum rise in plasma glucose of 12.7 ± 1.1 mmol l^{-1} ($P < 0.05$) occurred 60 min after the 50 μg kg^{-1} dose. This effect was significantly reduced but not abolished by pretreatment with naloxone (1 mg kg^{-1}, i.v.). After i.c.v. morphine (10 μg kg^{-1}) the maximum increase in plasma glucose was 7.9 ± 1.1 mmol l^{-1} ($P < 0.05$) after 60 min. There were no significant changes after i.c.v. morphine (1 μg kg^{-1}) or i.c.v. saline. In contrast, after i.c. morphine (50 μg kg^{-1}) there was a small gradual increase in plasma glucose reaching 3.9 ± 1.0 mmol l^{-1} ($P < 0.05$) above control after 120 min and this was prevented by pretreatment with i.v. naloxone (1 mg kg^{-1}) (Figure 2). There were no significant changes in plasma glucose after the 10 μg kg^{-1} and 1 μg kg^{-1} doses of i.c. morphine or after i.c. saline.

Effect of i.c.v. and i.c. morphine on respiration

Morphine given i.c.v. or i.c. caused a dose-related fall in respiration rate which led to an increase in $Paco_2$ and a fall in Pao_2 (Figure 3). Following morphine given via either route the maximum changes in respiration occurred after 90 min. At this time after the 50 μg kg^{-1} dose given i.c.v. (or i.c.) respiration rate had fallen by 120 ± 22 (142 ± 13) breaths min^{-1}. $Paco_2$ had increased by 22.9 ± 0.6 (23.2 ± 1.5) mmHg and Pao_2 had fallen by 25.8 ± 2.6 (35.7 ± 3.5) mmHg (Figure 3). At 90 min after 10 μg kg^{-1} morphine given i.c.v. (or i.c.) respiration rate had fallen by 116 ± 20 (143 ± 25) breaths min^{-1}, $Paco_2$ had risen by 22.4 ± 2.2 (18.7 ± 2.8) mmHg and Pao_2 had fallen by 30.7 ± 3 (24.7 ± 6) mmHg. The 1 μg kg^{-1} dose given i.c.v. (or i.c.) reduced respiration rate by 106 ± 14 (68 ± 20) breaths min^{-1}, increased $Paco_2$ by 9.2 ± 1.8 (5.7 ± 2) mmHg and reduced Pao_2 by 7.8 ± 1.4 (7.9 ± 2.5) mmHg. The changes in $Paco_2$ and Pao_2 following i.c.v. and i.c. morphine (50 and 10 μg kg^{-1}) were significantly different from those after saline. Naloxone (0.5 mg kg^{-1}, i.v.) given 120 min after morphine returned blood gases to control values within 5 min and significantly increased respiration rate above control when given after i.c.v. but not i.c. morphine (Figure 3).

Pretreatment with naloxone (1 mg kg^{-1}, i.v.) initially prevented the respiratory depression caused by i.c.v. and i.c. morphine (50 μg kg^{-1}) but after 60 min respiratory depression began to develop (Figure 3). After naloxone pretreatment, i.c.v. but not i.c. morphine caused an increase in respiration rate of 107 ± 25 beats min^{-1} ($P < 0.05$) after 5 min which was accompanied by a fall in $Paco_2$ (7.6 ± 4.1 mmHg) and a rise in Pao_2 (14.6 ± 2.6 mmHg).

Effects of i.c.v. and i.c. morphine on behaviour

Morphine (1 μg kg^{-1}) given i.c.v. produced a mild degree of sedation which lasted 1–2 h. The sedation following the 2 higher doses was deeper and longer lasting and these doses also produced catalepsy. These effects were prevented by pretreatment with naloxone. In initial studies a higher dose of morphine of 100 μg kg^{-1} was used, but when given i.c.v. or i.c. this dose caused excitation and rapid circling movements. Therefore, the highest dose was reduced to 50 μg kg^{-1}. After i.c. administration there was

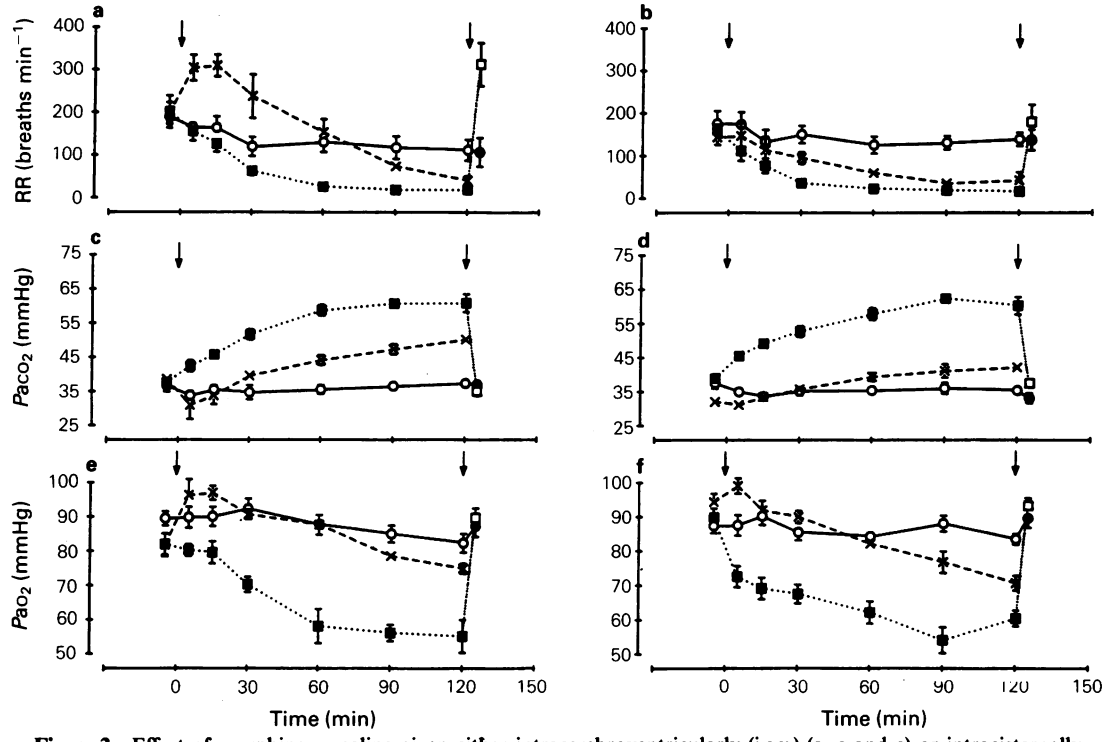

Figure 3 Effect of morphine or saline given either intracerebroventricularly (i.c.v.) (a, c and e) or intracisternally (i.c.) (b, d and f) on (a, b) respiration rate (RR), (c, d) $Paco_2$ and (e, f) Pao_2 in conscious rabbits. The effect of saline (100 μl) (○——○) or morphine (50 $\mu g\,kg^{-1}$) (■·····■) given at 0 min either i.c.v. ($n = 5$) or i.c. ($n = 6$) followed by i.v. naloxone (0.5 $mg\,kg^{-1}$) given 120 min after saline (●——●) or morphine (□·····□), and the effect of i.v. naloxone (1 $mg\,kg^{-1}$) given at −15 min on the response to i.c.v. ($n = 4$) and i.c. ($n = 5$) morphine (50 $\mu g\,kg^{-1}$) (×- - -×). The control samples were taken before the administration of drugs or vehicle. Each point represents the mean and vertical lines indicate s.e.mean.

only a mild degree of sedation which developed after the 50 $\mu g\,kg^{-1}$ dose.

Effect of ganglionic blockade on responses to i.c.v. morphine

In 5 conscious rabbits ganglionic blockade with i.v. pentolinium (5 $mg\,kg^{-1}$) reduced MAP from 93 ± 5 to 70 ± 3 mmHg and increased HR from 190 ± 8 to 226 ± 10 beats min^{-1} after 10 min. At 10 min after pentolinium, administration of i.c.v. morphine (50 $\mu g\,kg^{-1}$) had no cardiovascular effects. At 15 min after morphine, the time of the peak response, MAP (91 ± 8 mmHg) was similar to the control value and HR (244 ± 14 beats min^{-1}) remained elevated. After 60 min MAP (105 ± 9 mmHg) was not significantly different from control and the tachycardia (246 ± 14 beats min^{-1}) was unchanged. Pentolinium given alone to conscious rabbits causes a transient fall in MAP, a prolonged increase in HR and reduces plasma catecholamines (May *et al.*, 1989).

Ganglionic blockade abolished the increase in plasma noradrenaline and adrenaline that followed i.c.v. morphine (Table 1). The respiratory depressant effects of i.c.v. morphine were unaffected by pentolinium pretreatment, the changes in blood gases (Table 1) being similar to those after morphine alone (Figure 3).

Discussion

In these studies the effects of morphine given i.c.v. or i.c. to conscious rabbits have been compared. After i.c.v. morphine the changes were similar to those after systemic administration and included an increase in plasma catecholamines, hypertension, hyperglycaemia, respiratory depression and sedation. These effects were dose-related and occurred at considerably lower doses than required systemically (10 $\mu g\,kg^{-1}$ i.c.v. being equivalent to 4 $mg\,kg^{-1}$ i.v.).

Table 1 Effect of intracerebroventricular (i.c.v.) morphine ($50\,\mu g\,kg^{-1}$) on plasma catecholamines and respiration after pretreatment with i.v. pentolinium ($5\,mg\,kg^{-1}$) in 5 rabbits

		Time after morphine (min)			
	Control	5	10	30	60
Noradrenaline ($nmol\,l^{-1}$)	2.08 ± 0.60	2.00 ± 0.38	1.57 ± 0.23	2.09 ± 0.28	3.19 ± 0.57
Adrenaline ($nmol\,l^{-1}$)	0.36 ± 0.22	0.33 ± 0.22	0.51 ± 0.39	0.19 ± 0.11	0.33 ± 0.23
Respiration rate (breaths min^{-1})	166 ± 34	228 ± 19	208 ± 24	119 ± 34	39 ± 6
$Paco_2$ (mmHg)	34.3 ± 0.8	38.8 ± 1.1	42.6 ± 1.4	47.3 ± 0.9	53.4 ± 1.0
Pao_2 (mmHg)	82.4 ± 1.6	79.5 ± 3.0	71.4 ± 4.3	69.4 ± 2.3	66.1 ± 2.7

The second post treatment measurement of respiration rate and blood gases was made after 15 not 10 min.

In contrast, after i.c. morphine there was little effect on plasma catecholamines or BP, but the degree of respiratory depression and fall in HR were similar. These effects of morphine do not result from leakage into the peripheral circulation, as we have previously shown that a higher dose of morphine ($300\,\mu g\,kg^{-1}$) given i.v. had no effect (May *et al.*, 1988).

These findings are consistent with those of Conway *et al.* (1983) who demonstrated that in conscious rats i.c.v. morphine produced hypertension, catecholamine release and respiratory depression. The present finding that the morphine-induced increase in sympatho-adrenal activity and hypertension occurs after i.c.v. but not i.c. administration, suggests that it results from an action on periventricular structures and not from brainstem sites. A periventricular site of action for morphine is supported by the demonstration that in conscious rats microinjections into the hypothalamus of the μ-opioid agonist (D-Ala^2, MePhe^4, Gly^5-ol) enkephalin (DAGOL) increased plasma catecholamines and caused hypertension (Pfeiffer *et al.*, 1983).

The fall in HR after morphine appears to consist of two components. An initial fall concurrent with the rise in BP, that is probably baroceptor mediated, and a delayed fall similar to that after i.c. morphine. The bradycardia after i.c. morphine, which occurs in the absence of a significant rise in BP, may result from an increase in vagal tone due to stimulation of brainstem opioid receptors. Similar conclusions have been reached from studies in which i.c. administration of opiates caused bradycardia, although this was accompanied by hypotension probably because the studies were in anaesthetised animals (Laubie *et al.*, 1974; Feldberg & Wei, 1981). The tachycardia in response to naloxone, given 120 min after i.c.v. or i.c. morphine, is similar to that previously observed when naloxone was given after DAGOL (Pfeiffer *et al.*, 1983). This may result from antagonism by naloxone of an increase in vagal tone mediated by opioid receptors, probably in the brainstem as it occurs after i.c. as well as after i.c.v. morphine. The greater rise in the group treated with i.c.v. morphine may result from the positive chronotropic effects of the higher plasma levels of adrenaline in these animals at this time.

The cardiovascular effects of opiates could result from respiratory depression since hypoxia and hypercapnia increase sympatho-adrenal outflow (Korner & White, 1966). However, the differences in time course between the rapid increase in BP and the slowly developing respiratory depression argues against this as a mechanism. In addition, hypertension did not develop after i.c. morphine, although the degree of respiratory depression was similar, indicating that the initial increase in BP is independent of respiratory depression. However, the later rise in BP at 60 min after i.c.v. morphine, and the small rise in BP following i.c. morphine, may result from increased noradrenaline release secondary to the respiratory depression. The finding that i.c.v. morphine, given after naloxone pretreatment, caused hypertension in the absence of respiratory depression is further evidence that the morphine-induced increase in sympatho-adrenal outflow and rise in MAP are not caused by the hypoxia and hypercapnia.

In the present experiments the degree of hyperglycaemia was related to the increase in plasma adrenaline levels; plasma glucose was greater after i.c.v. than i.c. morphine and was reduced by naloxone pretreatment which reduced the rise in adrenaline. This agrees with the finding that in conscious rabbits the hyperglycaemic effect of i.v. morphine results from a centrally mediated increase in adrenaline secretion (May *et al.*, 1988; 1989). The greater rise after i.c.v. compared with i.c. morphine indicates an action on periventricular sites. However, in the cat the hyperglycaemic potency of i.c.v. morphine (0.75 mg) was similar to i.c. morphine (1.5 mg)

(Feldberg *et al.*, 1985). Hyperglycaemia was produced by injections into the subarachnoid space below the ventral surface of the brainstem which was proposed as the site of action of morphine. This species difference may be due to different localisation of μ-receptors in cat and rabbit or because the tissue distribution of morphine is different in the two species.

We have previously demonstrated that the hypertension following i.v. morphine results from increased sympatho-adrenal outflow (May *et al.*, 1989). The present findings indicate that this is also the mechanism by which i.c.v. morphine increases BP, as the increases in plasma catecholamines were similar and ganglionic blockade prevented the hypertension after i.v. and i.c.v. morphine. However, the central mechanisms leading to the increased sympatho-adrenal outflow appear to differ, as discussed below.

Naloxone, given i.v. ($1\,mg\,kg^{-1}$) or i.c.v. ($100\,\mu g\,kg^{-1}$), did not prevent the rise in plasma catecholamines or hypertension after i.c.v. morphine, although the respiratory depression and sedation were prevented. In contrast the effects of i.v. morphine are prevented by one tenth of these doses of naloxone, i.e. $100\,\mu g\,kg^{-1}$ given i.v. (unpublished) or $10\,\mu g\,kg^{-1}$ given i.c.v. (May *et al.*, 1989). These findings demonstrate that the increase in sympatho-adrenal outflow after i.c.v. morphine is naloxone-resistant which is consistent with previous observations. The hyperglycaemic responses to i.c.v. and i.c. morphine in conscious cats were not blocked by i.v. naloxone, although the effects of an equivalent dose of morphine given i.v. were blocked by the same dose of naloxone (Feldberg *et al.*, 1983). In conscious rats i.c.v. morphine increased BP and plasma catecholamines and these effects were not prevented by i.c.v. or i.v. naloxone (Conway *et al.*, 1983). These authors suggested that this could be explained by the different concentrations of agonists and antagonists at central receptor sites due to different routes of administration. However, this seems unlikely considering our demonstration that the hypertensive effects of i.c.v. morphine are not blocked by doses of naloxone, given either i.c.v. or i.v., which are 10 times greater than the doses needed to abolish the effects of i.v. morphine.

Naloxone can increase sympatho-adrenal outflow and blood pressure when given after various forms of shock (Holaday, 1983), which would obscure any inhibition of the effects of morphine. However, we have previously demonstrated that in unstressed conscious rabbits i.v. naloxone ($1\,mg\,kg^{-1}$) has no effect on systolic or diastolic blood pressure or on HR (May *et al.*, 1988). This is in agreement with other data demonstrating that opiate antagonism does not alter blood pressure or HR in conscious rabbits or rats (Holaday & Faden, 1978; Petty & Reid, 1982; Rutter *et al.*, 1987). The mechanism by which i.c.v. morphine increases sympatho-adrenal outflow awaits investigation but may involve central release of neurotransmitters or neuropeptides by morphine. Central administration of histamine elevates BP, probably by increasing sympathetic outflow as it is prevented by ganglionic blockade (Trendelenburg, 1957). In addition, several neuropeptides act centrally to alter sympatho-adrenal outflow (Brown & Fisher, 1984) which leads to selective cardiovascular changes.

In conclusion, these results indicate that the cardiovascular effects of centrally administered morphine result from actions on a number of sites. The increased sympatho-adrenal outflow appears to be mediated mainly via periventricular structures, but the mechanism appears to be different from that after i.v. morphine. The bradycardia is probably a combination of a reflex response and an increase in vagal tone, possibly mediated by brainstem opiate receptors. The respiratory depression is probably mediated by opiate receptors in the brainstem.

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