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Effects of μ-opioid receptor agonists on circulatory responses to simulated haemorrhage in conscious rabbits

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1 Cardiac output, arterial pressure, heart rate, systemic vascular conductance, respiratory rate and arterial blood P_{O_2} and P_{CO_2} were measured in unanaesthetized rabbits. Haemorrhage was simulated by inflating a cuff placed around the inferior vena cava so that cardiac output fell at a constant rate of about 8% of its resting value per min.

2 The effects of drug treatments on resting haemodynamic and respiratory variables, and on the haemodynamic response to simulated haemorrhage, were tested. The treatments were; 4th ventricular (−)-naloxone HCl (10–100 nmol), 4th ventricular H-Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO; 30–300 pmol), and i.v. morphine sulphate (0.5–5.0 μmol kg^{-1}). The interactions of graded 4th ventricular doses of naloxone (3–100 nmol) with the actions of DAMGO (100–300 pmol) on these responses were also assessed.

3 After sham treatments, the circulatory response to simulated haemorrhage had two phases. During the first compensatory phase, systemic vascular conductance fell, heart rate rose, and mean arterial pressure fell by only about 7 mmHg. A second decompensatory phase supervened when cardiac output had fallen by about 50%. At this point systemic vascular conductance rose abruptly and arterial pressure fell to $\leqslant$40 mmHg.

4 Low 4th ventricular doses of naloxone (10–30 nmol) and DAMGO (30–100 pmol) had no discernible effect on the circulatory response to simulated haemorrhage. Higher doses of naloxone (30–100 nmol) and DAMGO (100–300 pmol) prevented the decompensatory phase. These high doses of naloxone and DAMGO lowered resting heart rate without affecting the other haemodynamic or respiratory variables.

5 Low doses of i.v. morphine (0.5–1.5 μmol kg^{-1}) also had no discernible effect on the circulatory response to simulated haemorrhage. Higher doses of morphine (1.5–5.0 μmol kg^{-1}) abolished the decompensatory phase. These high doses caused respiratory depression without affecting the resting haemodynamic variables.

6 The prevention of circulatory decompensation by high doses of DAMGO was reversed by 3–10 nmol of naloxone in 3 out of 4 rabbits and by 10–30 nmol of naloxone in all 4 rabbits. The decompensatory phase was, however, prevented by the combined high doses of DAMGO (100–300 pmol) and naloxone (30–100 nmol).

7 These findings provide strong evidence that activation of μ-opioid receptors in the central nervous system abolishes circulatory decompensation during acute reduction of central blood volume in conscious rabbits. This effect does not appear to be due to activation of arterial chemoreceptors or to a non-specific increase in sympathetic vasoconstrictor drive, since respiratory depression and hypertension were not observed after 4th ventricular doses of DAMGO which abolished circulatory decompensation. Our results also provide indirect confirmation of our previous finding that naloxone acts to prevent circulatory decompensation by an antagonist action at central δ-receptors.

Introduction

During acute haemorrhage or simulated haemorrhage in the conscious rabbit, arterial pressure is initially maintained by an increase in peripheral resistance but when cardiac output has fallen by about 50% the compensatory vasoconstriction fails abruptly and arterial pressure plummets (Schadt *et al.*, 1984; Ludbrook & Rutter, 1988; Ludbrook *et al.*, 1988). The initial compensatory vasoconstriction is reflex in origin and is due principally to unloading of the arterial baroreceptors (Ludbrook & Graham, 1984). There is strong evidence that its subsequent failure is triggered by a signal from the heart (Burke & Dorward, 1988; Evans *et al.*, 1989a). There is also evidence, based on the relative abilities of selective antagonists injected into the 4th ventricle to prevent its occurrence, that it is mediated by an endogenous opioid mechanism within the central nervous system which involves δ-receptors (Evans *et al.*, 1989b).

We have also found, unexpectedly, that 4th ventricular administration of the selective μ-opioid receptor agonist H-Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (DAMGO) (Handa *et al.*, 1981) in a dose of 30 nmol prevents circulatory decompensation during simulated haemorrhage (Evans *et al.*, 1989b). We have argued that it is unlikely that this effect is mediated by blockade of δ-opioid receptors, since DAMGO is at least 500 times less potent than naloxone as a δ-antagonist (Sheehan *et al.*, 1986). The first goal of our present experiments was to test this proposition directly, by studying the interaction between DAMGO and the selective μ-receptor antagonist naloxone when they were both injected into the 4th ventricle.

When the μ-receptor agonist morphine (10–50 μg kg^{-1}) is injected into the lateral ventricle of the conscious rabbit it causes an increase in arterial pressure, associated with elevation of plasma concentrations of noradrenaline and adrenaline and with respiratory depression (May *et al.*, 1989b). This raises the possibility that 4th ventricular injection of DAMGO prevents circulatory decompensation during simulated haemorrhage because it raises sympathetic vasoconstriction drive and peripheral resistance as a result of its action on μ-receptors within the central nervous system, or because of

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the secondary effects of low arterial P_{O_2} and high P_{CO_2} on the arterial chemoreceptors. We have therefore determined the smallest dose of DAMGO administered to the 4th ventricle that would prevent circulatory decompensation during simulated haemorrhage, and measured its effects on systemic vascular conductance, respiratory rate and arterial blood gases.

We have also tested whether intravenous morphine would prevent circulatory decompensation in a dose which had no effects on the circulation or respiration, with the view of seeing whether the analgesic doses of morphine that are used in clinical practice would be likely to support arterial pressure in cases of acute blood loss.

Methods

Four New Zealand White rabbits, weighing 2.36–3.12 kg (mean 2.74), were used. The method of preparing the rabbits and the experimental protocols were similar to those described previously (Evans *et al.*, 1989a, b). The experiments were conducted in accordance with the Statement on Animal Experimentation by the National Health and Medical Research Council of Australia.

Surgical preparation

Rabbits were anaesthetized with halothane/oxygen after induction with intravenous thiopentone sodium (35–40 mg, Pentothal, Abbott) and endotracheal intubation. Buprenorphine HCl (60 μg s.c.; Reckitt & Colman) was administered post-operatively as an analgesic. Each rabbit underwent three separate operations. During the first operation an inflatable cuff was placed around the thoracic inferior vena cava (caval cuff). Two to three weeks later an electromagnetic flow probe (Biotronex BL5050) was placed around the ascending aorta. The flow probes had previously been calibrated *in vitro* by use of cellophane dialysis tubing and human whole blood with a haematocrit of 38% (Faris *et al.*, 1981). Two weeks later a polyvinyl chloride tube (Dural Plastics SV10), which had been heated and drawn out, was introduced through the atlanto-occipital ligament so that its 0.3 mm o.d. tip lay in the 4th ventricle. Its dead space of 15 μl was filled with 154 mM NaCl. The first study was done 10 days later.

Preparations for studies

These were done under local analgesia with 2% procaine HCl. The rabbit was placed in a 15 × 40 cm box fitted with a wire mesh lid, 60 min before the beginning of the study. The connecting plug for the flow probe and the ends of the tube and catheter were retrieved from their subcutaneous positions. Catheters were introduced into each central ear artery and advanced to the root of the ear, to measure arterial pressure and to collect arterial blood samples for blood-gas analysis. When necessary, a catheter was introduced into a marginal ear vein for i.v. drug administration.

Measurement of haemodynamic variables

The ear artery catheter was connected to a Statham P23Dc strain gauge zeroed 50 mm above the floor of the rabbit's box. The flow probe was connected to a Biotronex BL-613 meter to measure cardiac output. Heart rate was measured by a tachometer actuated by the flow pulse. Signals were amplified and recorded on a Grass Model 7 Polygraph.

The output from the Grass Polygraph was sent to an Olivetti M24 computer equipped with an analogue-to-digital converter which provided 10 s mean values of arterial pressure (MAP, mmHg), cardiac output (MCO, ml min^{-1}) and heart rate (MHR, beats min^{-1}). The computer also calculated 10 s means for cardiac index (MCI = MCO/body weight in kg) and systemic vascular conductance index (MSVCI = 10^2 MCI/MAP).

Respiration

A mercury-in-silastic strain gauge was placed around the thorax and connected to a bridge-circuit (Parks Electronics Laboratory, Oregon, U.S.A.), to monitor respiratory rate.

Haematocrit

Duplicate measurements (Hawksley) were made before each days' study from 0.2 ml samples of arterial blood. The haematocrit at the beginning of the first and last studies was 38.3 ± 0.6% and 35.0 ± 0.2% respectively.

Arterial blood gases

P_{aO_2} and P_{aCO_2} were measured on 0.6 ml samples of arterial blood, collected ~8 min after each drug treatment (Radiometer ABL 4 acid-base analyser, Copenhagen, Denmark).

Simulated haemorrhage

The caval cuff was inflated gradually by means of a micrometer syringe so that MCI fell at a rate of 11.97 ± 0.96 ml kg^{-1} min^{-1} per min (8.10 ± 0.11% of its resting level per min). The cuff was released when MAP had fallen to ~ 40 mmHg or when MCI had fallen to 36.8 ± 1.1% of its resting level.

Drugs

For 4th ventricular administration, (−)-naloxone HCl (Sigma) and DAMGO (Sigma) were dissolved in 154 mM NaCl at concentrations of 600 and 0.6 nmol per 15 μl respectively. For i.v. administration, morphine sulphate was used as a 10 mg ml^{-1} (15 mM) stock solution (David Bull Laboratories). All dilutions were made on the morning of the experiment, in 154 mM NaCl.

Experimental protocol

Each rabbit was studied four times at intervals of 2–7 days. In each study simulated haemorrhage (Ludbrook *et al.*, 1988) was repeated 3–5 times at intervals of not less than 90 min. When a drug was given into the 4th ventricle, the loading dose was injected in a volume of 15 μl over 1 min, 10 min before the start of simulated haemorrhage. This was followed by infusion of the same concentration at 0.75 μl min^{-1} until the caval cuff was deflated. A similar protocol was followed for i.v. administration of morphine, except that the loading dose was given in a volume of 1 ml kg^{-1} and was followed by an infusion at 50 μl kg^{-1} min^{-1}.

We first determined the lowest doses of the μ-opioid agonist DAMGO, and of the μ-selective opioid antagonist naloxone, which abolished circulatory decompensation during simulated haemorrhage. Thus in the first two studies, performed in random order, we examined the effects of 4th ventricular administration of ascending doses of naloxone (0, 10, 30, and in two cases 100 nmol) and of ascending doses of DAMGO (0, 30, 100, and 300 pmol). Given this information, we set out to determine what dose of naloxone would antagonize the effects of DAMGO on the haemodynamic response to simulated haemorrhage. We also tested whether there was an i.v. dose of the μ-receptor agonist morphine that would prevent circulatory decompensation during simulated haemorrhage, without causing respiratory depression. These second two aims were realized in the third and fourth studies, performed in random order. In the third study, the effects of the lowest effective 4th ventricular dose of DAMGO (determined from the second study) were re-tested, then the effects of combining this dose of

DAMGO with ascending 4th ventricular doses of naloxone (10, 30 and 100% of its effective dose determined in the first study). In the fourth study, the effects of ascending doses of i.v. morphine (0.5, 1.5 and 5.0 μmol kg^{-1}) were tested. In each study the effects of injecting saline vehicle, by the 4th ventricular or i.v. route, were tested first (sham treatments).

Autopsy

At the conclusion of the experiments the rabbits were killed by intravenous administration of pentobarbitone sodium (Nembutal, Ceva Chemicals). Autopsy showed that in all four rabbits the flow probe was firmly bonded to the aorta, and that the tip of the 4th ventricular catheter was properly positioned.

Statistical analysis

Analysis of data was carried out as described by Evans *et al.* (1989a, b).

Analysis of variance (ANOVA) was used to evaluate the effects of treatments on the levels of the haemodynamic and respiratory variables. The factors included rabbits, studies, drug treatments within studies, and before/after drug administration. Two separate analyses were performed. The data were first categorized by dose of drug. The interaction between dose of drug and the before/after levels of the haemodynamic variables, and the main effect of dose on the levels of the respiratory variables, were used to test for dose-dependent effects. In a second analysis, the haemodynamic and respiratory data for 4th ventricular naloxone, DAMGO and DAMGO-naloxone mixtures, and for i.v. morphine, were categorized according to sham treatments, treatments which did not abolish the decompensatory phase of the response to simulated haemorrhage, and the lowest doses that did abolish the decompensatory phase. For each variable, specific contrasts were made within the ANOVA between the levels after doses which did not prevent circulatory decompensation or those that did, and the level after sham treatment. The critical value of F was adjusted by means of the Bonferroni correction.

Regression analysis confirmed that MCI, expressed as a percentage of its pre-treatment level (%MCI), fell linearly with time during simulated haemorrhage ($r = 0.979 \pm 0.003$). As we have found previously (Evans *et al.*, 1989a), there was a near-linear relationship of MAP, MHR and MSVCI to %MCI during simulated haemorrhage, up to the point that the abrupt rise of MSVCI occurred. This enabled us to use the same graphical method for analysing the effects of opioid agonists and antagonists on the responses of MSVCI, MAP and MHR to simulated haemorrhage as we have used previously (Evans *et al.*, 1989a, b). The relationships of MAP, MHR and MSVCI to %MCI were characterized by three sets of co-ordinates: (a) Post-treatment levels, immediately before the onset of simulated haemorrhage; (b) the point at which MSVCI reached a minimum, before it rose abruptly; and (c) the final observation before the caval cuff was deflated. These co-ordinates were averaged, first within and then between rabbits, so as to distinguish the effects of sham treatments, drug treatments that did not abolish the decompensatory phase of the response to simulated haemorrhage, and those that did.

Unless otherwise indicated, the levels of the variables are tabulated as between rabbit means ± s.e.mean.

Balance of the experiment

The design of the experiment was, of necessity, only partly balanced. Although each rabbit underwent all four studies, the effects of 4th ventricular naloxone and DAMGO were always tested in the first two studies, and the 4th ventricular DAMGO-naloxone interaction and the effects of i.v. morphine in the second two studies. Also, the order of doses within each study was fixed. For these reasons we tested for possible sources of bias.

Among the four studies there was a suggestion of heterogeneity among the levels of MCI prior to sham treatment ($F = 4.1$; d.f. 3, 9; $P = 0.04$), but none was detectable in the cases of MAP, MHR, or MSVCI (P always > 0.2). Among the four studies there was no heterogeneity of respiratory rate, Pa_{O_2} or Pa_{CO_2} after sham treatment (F always < 3.5; d.f. 3, 9; P always > 0.05). The rate of fall of MCI during simulated haemorrhage was independent of studies and of treatments within studies (P always > 0.05).

Results

Effects of drug treatments on levels of haemodynamic and respiratory variables

At the beginning of each study the levels of the haemodynamic variables were within the normal range for our laboratory (Evans *et al.*, 1989a, b) (Figure 1). None of the drug treatments affected the levels of MCI, MAP or MSVCI but some affected MHR (Figure 1). Doses of naloxone (30–100 nmol) and DAMGO (100–300 pmol) that abolished the decompensatory phase of the response to simulated haemorrhage (see below), lowered MHR by 39 ± 12 and 29 ± 6 beats

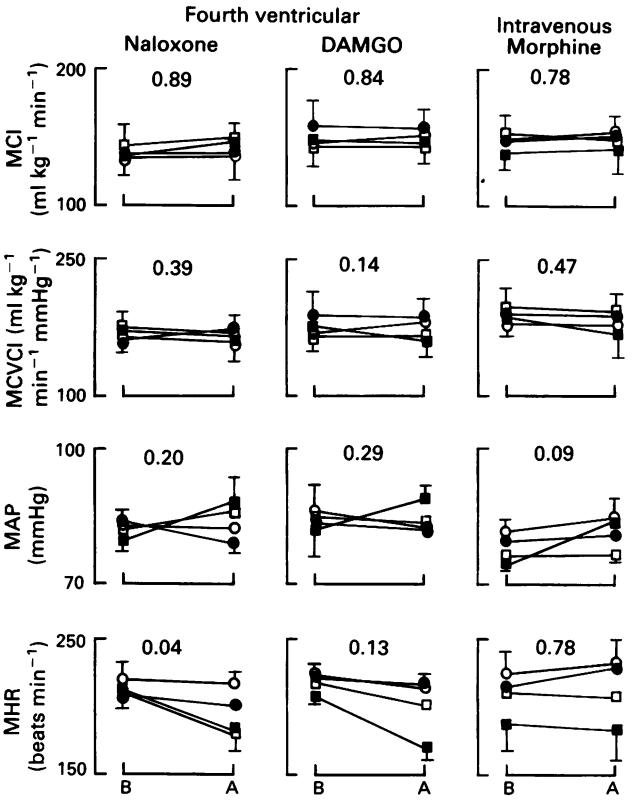

Figure 1 Effects on resting haemodynamic variables of 4th ventricular naloxone (○, saline vehicle; ●, 10 nmol; □, 30 nmol; ■, 100 nmol), 4th ventricular DAMGO (○, saline vehicle; ●, 30 pmol; □, 100 pmol; ■, 300 pmol), and i.v. morphine (○, saline vehicle; ●, 0.5 μmol kg^{-1}; □, 1.5 μmol kg^{-1}; ■, 5.0 μmol kg^{-1}). Values are 1 min averages for immediately before injection (B) and 9 min after injection (A). MCI = mean cardiac index; MSVCI = mean systemic vascular conductance index; MAP = mean arterial pressure; MHR = mean heart rate. Each point is the mean of 4 observations (except for the 100 nmol dose of naloxone, where $n = 2$). Vertical bars represent s.e.mean. Numbers on the top of each panel are P values for the interaction between dose and before/after drug administration (d.f. = 3,21 except for naloxone where d.f. = 3,17). Note the effect of naloxone on MHR.

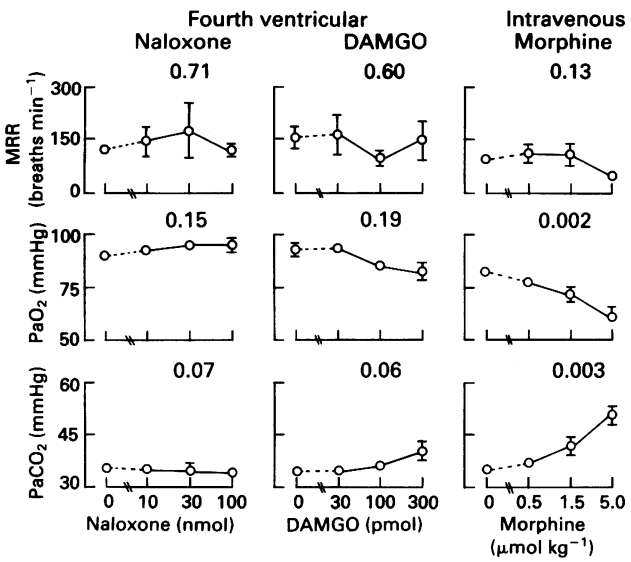

Figure 2 Effects on resting respiratory variables of 4th ventricular naloxone (0, 10, 30 and 100 nmol), 4th ventricular DAMGO (0, 30, 100 and 300 pmol), and i.v. morphine (0, 0.5, 1.5 and 5.0 μmol kg^{-1}). MRR = mean respiratory rate; Pao_2 = oxygen partial pressure of arterial blood; $Paco_2$ = carbon dioxide partial pressure of arterial blood. Each point is the mean of 4 observations (except for the 100 nmol dose of naloxone where $n = 2$) made ~8 min after injection. Vertical bars represent s.e.mean. Where no vertical bars are shown, s.e.mean is less than the symbol radius. Values on the top of each panel are P values for the main effect of dose (d.f. = 3,9 except for naloxone where d.f. = 3,7). Note the effect of morphine on Pao_2 and $Paco_2$.

min^{-1} respectively. When these doses of DAMGO and naloxone were combined, MHR was lowered by 35 ± 9 beats min^{-1} ($P < 0.01$).

The respiratory variables were also within a normal range at the beginning of each study (Figure 2). Respiratory rate was not affected by any of the treatments (Figure 2). The arterial blood gas tensions were affected only by i.v. morphine. Doses which abolished the decompensatory phase of the response to simulated haemorrhage (1.5–5.0 μmol kg^{-1}) lowered Pao_2 to 68 mmHg and raised $Paco_2$ to 46 mmHg.

Effects of 4th ventricular DAMGO or naloxone, and of i.v. morphine, on the haemodynamic responses to simulated haemorrhage

After the sham treatments, the haemodynamic response to simulated haemorrhage was always biphasic (Figure 3). During the first, compensatory, phase MSVCI fell progressively and MHR rose, while MAP fell by a grand mean of only 7 ± 2 mmHg. The second, decompensatory, phase began when MCI had fallen to 52 ± 1% of its pre-treatment level, at which stage MSVCI rose abruptly and MAP fell precipitately. The caval cuff was deflated when MAP had fallen to 37 ± 1 mmHg and MCI was 41 ± 1% of its pre-treatment level.

Low doses of naloxone (10–30 nmol) had no discernible effect on the haemodynamic responses to simulated haemorrhage. Higher doses (30–100 nmol) abolished the decompensatory phase (Figure 3). That is, throughout simulated haemorrhage there was a steady fall of MSVCI and rise of MHR. When the caval cuff was deflated, MAP had fallen by only 17 ± 2 mmHg, yet MCI was 35 ± 2% of its pre-treatment level (Figure 3).

Low doses of DAMGO (30–100 pmol) had no discernible effect on the haemodynamic response to simulated haemorrhage. Higher doses (100–300 pmol) abolished the decompensatory phase. When the caval cuff was deflated, MAP had fallen by only 7 ± 3 mmHg, yet MCI was 33 ± 3% of its pre-treatment level (Figure 3).

Low doses of i.v. morphine (0.5–1.5 μmol kg^{-1}) did not affect the haemodynamic response to simulated haemorrhage, but higher doses (1.5–5.0 μmol kg^{-1}) abolished the decompensatory phase. When the caval cuff was deflated, MAP had fallen by only 11 ± 3 mmHg, yet MCI was 35 ± 3% of its pre-treatment level (Figure 3).

Interaction between effective doses of DAMGO and ascending doses of naloxone

After sham treatment, the haemodynamic response to simulated haemorrhage followed the typical biphasic pattern (Figure 4). During the first, compensatory, phase MSVCI fell progressively and MHR rose, while MAP fell by only 9 ± 4 mmHg. The second, decompensatory, phase began when MCI had fallen to 50 ± 2% of its pre-treatment level, at which stage MSVCI rose abruptly and MAP fell precipitately. The caval cuff was deflated when MAP had fallen to 38 ± 1 mmHg and MCI was 38 ± 3% of its pre-treatment level.

As was to be expected, the previously-determined effective dose of DAMGO (100–300 pmol) abolished the decompensatory phase of simulated haemorrhage (Figure 4). When the caval cuff was deflated, MAP had fallen by only 3 ± 3 mmHg yet MCI was 36 ± 1% of its pre-treatment level.

When 10% of the effective dose of naloxone (3–10 nmol) was combined with the effective dose of DAMGO, circulatory decompensation occurred during simulated haemorrhage in three of the four rabbits. When 30% of the effective dose of naloxone (10–30 nmol) was combined with DAMGO, circulatory decompensation occurred during simulated haemorrhage in all four rabbits (Figure 4). However, when the maximum dose of naloxone (30–100 nmol) was combined with DAMGO,

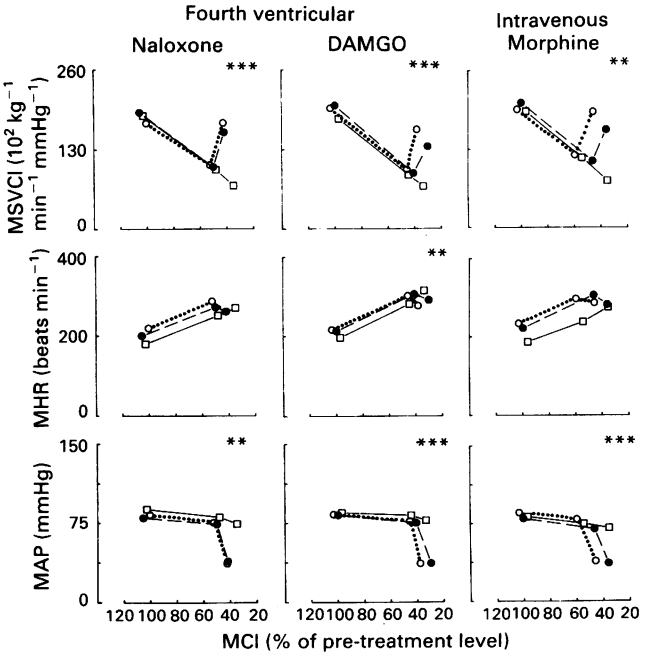

Figure 3 Haemodynamic variables during simulated haemorrhage after 4th ventricular naloxone, 4th ventricular DAMGO, and i.v. morphine. Haemodynamic variables as for Figure 1. Lines join average co-ordinates for 4 rabbits. The order of treatments was: (○·····○) saline vehicle; (●– – – –●) low doses which did not prevent circulatory decompensation; (□————□) high doses which did prevent circulatory decompensation. Low doses of naloxone, DAMGO and morphine were respectively 10–30 nmol, 30–100 pmol and 0.5–1.5 μmol kg^{-1}. High doses were respectively 30–100 nmol, 100–300 pmol and 1.5–5.0 μmol kg^{-1}. Outcome of ANOVA at d.f. 1,6 for the effects of high doses on haemodynamic variables at the end of simulated haemorrhage: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

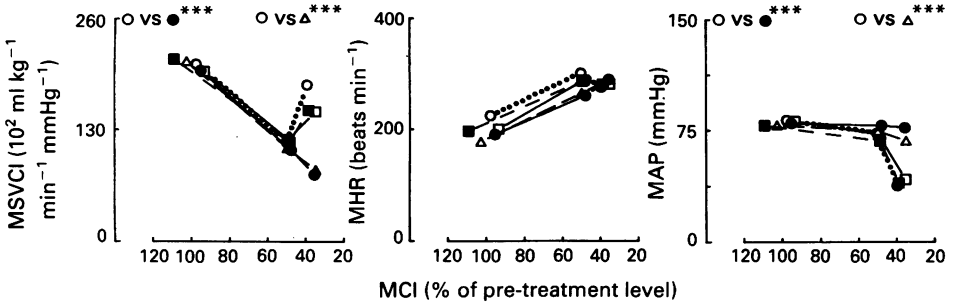

Figure 4 Haemodynamic changes during simulated haemorrhage after 4th ventricular administration of saline vehicle, the lowest dose of DAMGO which prevents circulatory decompensation, or a DAMGO-naloxone mixture. Haemodynamic variables as for Figure 1. Lines join average co-ordinates for 4 rabbits. The order of treatments (and the loading doses) were: (○·····○) saline vehicle; (●———●) the lowest effective dose of DAMGO (100–300 pmol); (□- - - -□) the lowest effective dose of DAMGO plus 10% of the lowest effective dose of naloxone (3–10 nmol); (■- - - -■) the lowest effective dose of DAMGO plus 30% of the lowest effective dose of naloxone (10–30 nmol); (△- - - -△) the lowest effective dose of DAMGO plus 100% of the lowest effective dose of naloxone (30–100 nmol). Contrasts were made within ANOVA at d.f. 1,12 between levels at the end of simulated haemorrhage after saline treatment and after each of the active treatments. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

the decompensatory phase did not occur (Figure 4). When the caval cuff was deflated, MAP had fallen by only 11 ± 3 mmHg, yet MCI was $35 \pm 1\%$ of its pre-treatment level.

Discussion

We have confirmed our earlier finding that small doses (30–100 nmol) of the opioid receptor antagonist (−)-naloxone HCl, when injected into the 4th ventricle, abolish the decompensatory phase of simulated haemorrhage in conscious rabbits (Evans *et al.*, 1989a, b). We have previously provided evidence that this effect of naloxone is mediated by an antagonist action at central δ-receptors (Evans *et al.*, 1989b). We have also extended our finding that circulatory decompensation is abolished after 4th ventricular administration of 30 nmol of the μ-agonist DAMGO (Evans *et al.*, 1989b), by demonstrating that the threshold dose for this effect is as low as 100–300 pmol. We now present direct evidence that this action of DAMGO is mediated by a μ-opioid receptor mechanism within the central nervous system, and that it is independent of effects on vasoconstrictor drive, respiratory rate or arterial blood gas tensions. We have also shown that intravenous morphine will abolish the decompensatory phase of simulated haemorrhage, but only in doses that cause respiratory depression.

The action of 4th ventricular DAMGO on the response to simulated haemorrhage

Naloxone exhibits selectivity as an antagonist at μ-receptors, being some 7–30 times less potent as a δ-antagonist and 10–15 times less potent as a κ-antagonist (Magnan *et al.*, 1982; North, 1986; Smith, 1987). In view of our previous work (Evans *et al.*, 1989b), it can be assumed that when 4th ventricular naloxone prevents the decompensatory phase of simulated haemorrhage it does so by blocking δ-opioid receptors. At lower doses, it should still block μ-receptors. DAMGO is a highly specific agonist at μ-receptors, having negligible activity at κ- and δ-receptors (Handa *et al.*, 1981; Miller *et al.*, 1986). We found that 10% of the dose of naloxone which prevented circulatory decompensation during simulated haemorrhage nullified the action of DAMGO in preventing circulatory decompensation in three out of four rabbits, and 30% did so in all four rabbits (Figure 4). We interpret this as showing that the action of 4th ventricular DAMGO in preventing the decompensatory phase of simulated haemorrhage is mediated by its agonist action at μ-receptors within the central nervous system. We also found that when the entire effective dose of naloxone was combined with DAMGO, the decompensatory phase was again prevented (Figure 4). We assume that this was because the dose of naloxone was then sufficient to block δ-receptors.

In our original report that 4th ventricular DAMGO prevented the decompensatory phase of simulated haemorrhage, we used an arbitrary dose of 30 nmol (Evans *et al.*, 1989b). This caused a fall in systemic vascular conductance and a rise of arterial pressure, and it appeared to slow respiration. The effective dose-range of DAMGO that we have now established is between 100 and 300 times less, and this had no significant effect on any of the haemodynamic or respiratory variables. Indeed, in every instance, Pa_{O_2} remained well above the threshold of 50 mmHg at which arterial chemoreceptor reflex effects on the circulation can be detected in conscious rabbits (Korner, 1965). This seems to exclude an alteration in baseline vasoconstrictor drive, or excitation of arterial chemoreceptors, as the basis for the action of DAMGO in preventing circulatory decompensation.

Does activation of μ-receptors within the central nervous system occur as part of the normal response to simulated and, by extension, actual haemorrhage? We have no evidence that suggests this is so. In particular, sub-critical doses of naloxone, which would be expected to block μ- but not δ-receptors, do not appear to affect the pattern of haemodynamic response to simulated haemorrhage (Figure 3; Evans *et al.*, 1989a, b). There is some evidence, however, for an interaction between central δ- and μ-receptors in the pathogenesis of endotoxic shock. Administration of the δ-antagonist M-154,129 reversed the hypotension induced by i.v. endotoxin injection in conscious rats (Holaday *et al.*, 1982). This effect was attenuated by prior administration of the irreversible μ-antagonist β-funaltrexamine (D'Amato & Holaday, 1984). The authors proposed an allosteric interaction between μ- and δ-binding sites to explain these findings.

Intravenous administration of the μ-agonist morphine also abolished the decompensatory phase of the response to simulated haemorrhage, at threshold doses of 1.5–5.0 μmol kg^{-1} (Figure 3). These intravenous doses of morphine always caused respiratory depression (Figure 2). This suggests that, in clinical practice, systemic administration of morphine is unlikely to extend the compensatory phase of haemorrhage unless the dose is so high as to cause respiratory depression.

Is there any information about the localization within the central nervous system of these δ- and μ-receptors which modulate the haemodynamic response to simulated haemorrhage? It is reasonable to assume that when drugs are injected into the 4th ventricle their concentrations are highest in the dorsal part of the pons and medulla. Within this region lies the nucleus tractus solitarii, an important relay station for afferents from both arterial baroreceptors and cardiac receptors (Jordan & Spyer, 1986). Recent work has shown that, in

the rabbit, there is a dense population of δ-binding sites within this nucleus (May *et al.*, 1989a). It is not so clear where μ-agonists, injected into the 4th ventricle, might act to prevent circulatory decompensation during simulated haemorrhage. There are dense populations of μ-binding sites in the nucleus tractus solitarii of the rat (Mansour *et al.*, 1988) and cat (Sales *et al.*, 1988; Dashwood *et al.*, 1988). In the rabbit, μ-binding sites have been found in areas adjacent to the nucleus tractus solitarii (May *et al.*, 1989a). The cardiovascular actions of intravenous morphine were always associated with respiratory depression, whereas the corresponding actions of 4th ventricular DAMGO were not. This difference is more likely to be a function of the route of administration than of the pharmacodynamics of these compounds. There is good evidence that intravenous morphine exerts its respiratory depressant action in the hindbrain (Florez *et al.*, 1968; May *et al.*, 1989a). The development of respiratory depression is much slower when morphine is injected into the 4th ventricle, cisterna magna, or pontomedullary cistern than when it is given intravenously (Florez *et al.*, 1968; May *et al.*, 1989a). Our findings therefore suggest that the cardiovascular neurones which are sensitive to μ-agonists are located near the dorsal surface of the pontomedullary region, whereas the μ-sensitive respiratory neurones are situated more deeply. The precise identification of the sites at which δ-antagonists and μ-agonists act to prevent circulatory decompensation during simulated haemorrhage, and at which μ-agonists act to cause respiratory depression, will probably rely on micro-injection studies.

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