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THE EFFECT OF INTRACEREBROVENTRICULAR INJECTIONS OF MORPHINE ON VASOPRESSIN RELEASE IN THE RAT

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SUMMARY

1. An investigation was carried out to determine the effect of intracerebroventricular (I.C.V.) micro-injections of morphine on vasopressin (AVP) release in the urethane-anaesthetized rat.

2. Plasma AVP levels at different time intervals, following I.C.V. injection of 10–150 μg morphine, were measured by radioimmunoassay. The effect of I.C.V. micro-injections of morphine on urine outflow was also studied in a group of water-loaded rats.

3. The vasopressin response to I.C.V. micro-injections of morphine was both dose- and time-dependent. High does of 50 and 150 μg morphine produced short latency stimulation of AVP release, followed by a fall. The low dose of 10 μg morphine produced only a long latency inhibition. The most consistent response of I.C.V. injection of morphine was an inhibition of release.

4. Both stimulatory and inhibitory effects of morphine on vasopressin release were naloxone reversible and stereospecific.

5. I.C.V. micro-injections of morphine produced a dose-dependent rise in mean arterial blood pressure of short latency. Naloxone (0·5 mg/kg) completely abolished the rise seen with 10 μg morphine and diminished the rise with 50 μg.

6. Doses of 10 and 50 μg morphine injected I.C.V. produced an immediate antidiuresis in water-loaded rats under urethane anaesthesia.

7. The vasopressin response to I.C.V. micro-injections of morphine is independent of the effects on the cardiovascular system and may involve different opiate receptor populations. The results also suggest the possibility that opiate receptors with different affinities for morphine may be responsible for the stimulatory and inhibitory effects of morphine on vasopressin release.

INTRODUCTION

The effect of morphine on vasopressin release has been a topic of much investigation and controversy for over five decades. As early as 1926 Scott & Loucks observed difficulty in producing a water diuresis in the decerebrate dog under morphine and chloroform–ether anaesthesia and Fee (1929) demonstrated that the antidiuretic response was due to morphine. Then in 1944 de Bodo observed antidiuresis following

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systemic morphine in the dog which appeared to be mediated by the liberation of vasopressin from the neurohypophysis. Recently there has been a resurgence of interest in this field with the realization that the pharmacological action of morphine may be mimicking the physiological activities of the naturally occurring neuropeptides, the enkephalins and endorphins. The location of stereospecific opiate receptors in posterior pituitary membranes by Simantov & Snyder in 1977 and the recent demonstration of an enkephalinergic pathway from the supraoptic nucleus to the posterior pituitary (Rossier, Battenberg, Pittman, Bayon, Koda, Miller, Guillemin & Bloom, 1979) are suggestive of a possible role for the endogenous opioids in controlling vasopressin secretion. There is overwhelming evidence in both conscious and anaesthetized preparations that morphine does have an effect on water balance in various species. However, its precise action on vasopressin release is not clear. The effect of intracerebroventricular (I.C.V.) micro-injections of morphine on vasopressin release in the rat has been studied by various groups, and conflicting results have been produced. In 1978 Bisset, Chowdrey & Feldberg observed an antidiuretic response to I.C.V. micro-injections of morphine in water-loaded ethanol-anaesthetized rats. On the other hand van Wimersma Greidanus, Thody, Verspaget, De Rotte, Goedemans, Croiset & van Ree in 1979 observed a decrease in plasma arginine vasopressin (AVP) concentration following I.C.V. injections of morphine in conscious dehydrated and salt-loaded rats.

The present investigation has been carried out with the aim of resolving this problem by studying the action on vasopressin release of I.C.V. injections of morphine in different doses and at various time intervals. For this study a sensitive and specific radioimmunoassay was employed for measuring AVP directly, and in addition we also investigated the antidiuretic response to I.C.V. micro-injections of different doses of morphine in the water-loaded, anaesthetized rat. A preliminary report of part of this study has been presented to The Physiological Society (Aziz, Forsling & Woolf, 1980).

METHODS

This study comprised two experimental approaches. Plasma AVP concentrations in the first groups of urethane-anaesthetized rats were measured at different times in response to I.C.V. micro-injections of morphine in varying doses. The second series of experiments involved monitoring the urine flow in a group of water-loaded urethane-anaesthetized rats given I.C.V. micro-injections of different doses of morphine.

I.C.V. micro-injections of morphine and plasma AVP

The experiments were performed on male Sprague–Dawley rats weighing 225–275 g which were given a water load of 24 ml./kg body weight by oro-gastric tube, and anaesthetized with an intraperitoneal injection of urethane in a dose 1·5 g/kg body weight. The trachea was cannulated and polythene catheters (bore 0·58 mm, external diameter 0·96 mm) placed in the carotid artery and jugular vein. A stainless steel cannula (27 g) was inserted stereotaxically into the third ventricle. The position of the intracerebral cannula was confirmed at the end of the experiment by a micro-injection of Evans blue dye and observing the distribution of the dye in the ventricular system. Twenty minutes after introduction of the cannula into the third ventricle, 0·8 ml. arterial blood was withdrawn for estimation of initial circulating AVP concentration. After a further 20 min morphine was injected I.C.V. in doses of 10–150 μg in 5 μl. and blood samples taken at either 1 and 3 min, 5 and 10 min or 20 and 30 min. An intravenous infusion of 0·15 M-NaCl at a rate of 0·1 ml./min was given throughout the experiment and the arterial blood pressure recorded using a pressure transducer (Bell & Howell, England). The haematocrit was determined for all blood samples.

A series of control experiments was performed in which 5 μl. physiological saline alone was injected into the third ventricle as well as a further series in which rats were given an intravenous injection of the morphine antagonist naloxone in a dose 0·5 mg/kg body weight 2 min prior to I.C.V. injection of morphine or saline. The effect of urethane anaesthesia alone on plasma vasopressin was characterized in a group of rats from which blood was obtained by decapitation.

Plasma vasopressin was determined by radioimmunoassay after prior extraction by adsorption to Bentonite, the recovery being 70% (Lightman & Forsling, 1980). Hormone levels were determined using synthetic AVP (400 i.u./mg, Ferring AB, Malmo, Sweden) as the standard, radioiodinated AVP as the label, prepared by the lactoperoxidase method of iodination (Karonen, Morsky, Siren & Seuderling, 1975), and an antiserum specific for AVP (cross-reactivity with LVP 10%, oxytocin 0·001%). The lowest limit of detection of the assay was 0·3 μu./ml. plasma.

I.C.V. micro-injections of morphine and urine outflow

For investigation of the effect of I.C.V. injections of morphine on urine outflow, a cannula was chronically implanted in the third ventricle of each of ten rats. A guide cannula (27 g) was stereotaxically positioned 1 mm above the third ventricle with the animal under sodium pentobarbitone anaesthesia (Sagatal, dose 40 mg/kg body weight). The cannula was fixed in position with dental acrylic cement and the cannula sealed with a sterile obturator. The rats were allowed a recovery period of up to 9 days before the experiments were performed. On the day of the experiment, the rats were anaesthetized with intraperitoneal injection of urethane (1·5 g/kg body weight) and an initial water load of 5 ml. given by stomach tube. After 30 min prior to dissection, a further 5 ml. tap water was given. A catheter was inserted into the left jugular vein, and when necessary the trachea was cannulated. The bladder was exposed, and a catheter introduced to allow urine output to be continuously recorded with a drop recorder and Devices pen recorder. Within 2 hr of the initial water load the rats produced a steady flow of 0·13–0·2 ml./min. Hydration was maintained by continuous infusion of 0·1 M-NaCl infused at a rate of 0·15 ml./min. Anaesthesia was supplemented when necessary by intravenously administered doses. Micro-injections of 10 and 50 μg morphine were given I.C.V. via an injection cannula (30 g) which was fed down the guide cannula into the third ventricle, and the response matched by the infusion of vasopressin at rates 2·5–40 μu./min.

Statistical analysis

The results are presented as the mean $\pm$ S.E. of mean and values were compared using the paired and unpaired t test where appropriate. If the P value was greater than 0·05 the difference between two groups was not regarded as significant.

RESULTS

The effect of I.C.V. micro-injections of morphine on plasma AVP

The initial plasma AVP concentration in the urethane-anaesthetized rat after surgery was elevated, being 9·8 $\pm$ 1·2 μu./ml. as compared to 1·1 $\pm$ 0·33 μu./ml. in the conscious rat. This elevation may be accounted for by the combined effect of anaesthesia and surgical trauma. Urethane itself was found to have a slightly stimulatory effect, producing a concentration of 5·2 $\pm$ 1·23 μu./ml.

The AVP response to I.C.V. injection of increasing doses of morphine at various time intervals is shown in Fig. 1. Injections of 50 and 150 μg morphine (I.C.V.) initiated a rapid release of vasopressin. Following injection of 50 μg morphine, two out of five rats produced an increase in plasma AVP at 1 min, while all five showed a rise at 3 min. Upon injection of 150 μg morphine, all four rats showed a small but not statistically significant stimulation at 1 min. Following I.C.V. injections of morphine at 50 and 150 μg, plasma AVP rose significantly, reaching peak values of 27·5 $\pm$ 9·9 μu./ml. and 34·3 $\pm$ 7·9 μu./ml. respectively at 10 min. At 10 min there was no detectable change in AVP level with injections of 10 μg morphine or 5 μl. saline. However, by 30 min

AVP levels had fallen significantly to below initial values for all doses of morphine. The plasma AVP levels at 30 min post-morphine were significantly different both from the initial AVP concentration in rats receiving morphine and from the concentrations in saline controls at 30 min, which had shown a rise on plasma AVP. This fall in AVP supports the observation of van Wimersma Greidanus *et al.* (1979).

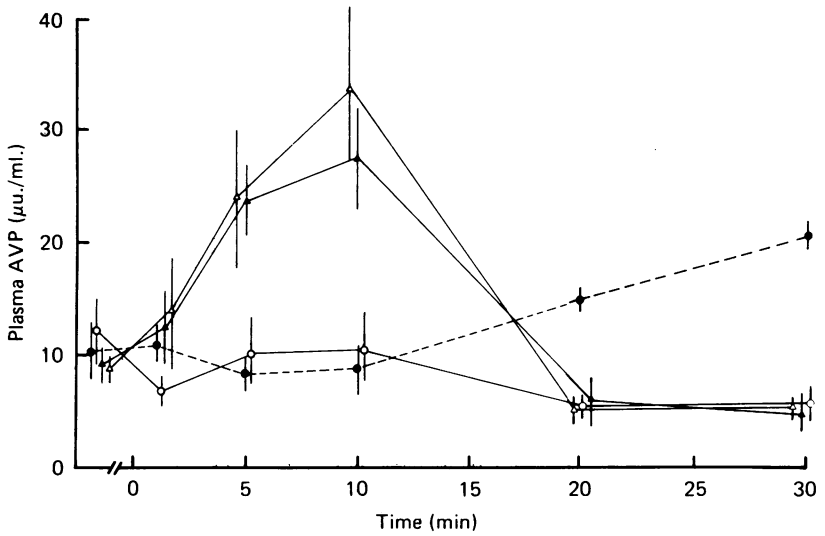

Fig. 1. The effect on plasma vasopressin concentrations of doses of 10 μg (○), 50 μg (▲) and 150 μg (△) morphine given intracerebroventricularly. The saline controls are represented by the closed circles and dashed line. Each value represents the mean of six observations, vertical bars indicate S.E.

Fig. 2 shows the dose–response curves at 1, 10 and 30 min for I.C.V. morphine. The 1 min curve shows no significant change in plasma AVP with any of the doses employed. The 10 min curve shows no change with micro-injections of 10 μg morphine, but significant increases with the higher doses of 50 and 150 μg. The 30 min curve shows a significant suppression of plasma AVP over the entire dose range.

When naloxone alone was injected intravenously at a dose of 0·5 mg/kg body weight, 2 min prior to I.C.V. injection of 5 μl. physiological saline none of the parameters measured differed significantly from those in the saline controls at the various time intervals studies. However, both the stimulatory and inhibitory effects of I.C.V. micro-injections of morphine on vasopressin release were reversed by the naloxone. The rise in plasma AVP concentration of $27{\cdot}5 \pm 4{\cdot}9$ μu./ml. produced at 10 min by 50 μg morphine injected I.C.V. was blocked, the plasma AVP concentration being $7{\cdot}8 \pm 1{\cdot}4$ μu./ml. Also, the suppression of plasma AVP to $6{\cdot}2 \pm 1{\cdot}1$ μu./ml. seen 20 min after the injection of 10 μg morphine was not seen when the morphine injection was preceded by intravenously administered naloxone, the plasma concentration being $16{\cdot}6 \pm 1{\cdot}7$ μu./ml.

The effect of I.C.V. micro-injections of the inactive enantiomer of morphine, (+) morphine, was studied in four rats. Two rats given 10 μg (+) morphine showed no

suppression of plasma AVP at 20 and 30 min. Also, 150 μg (+) morphine given to two rats produced no change in AVP levels at 5 and 10 min. These results indicate that the vasopressin response to I.C.V. micro-injections of morphine is stereospecific.

Micro-injections of morphine given I.C.V. produced a dose-dependent and significant increase in mean arterial blood pressure of short latency. Fig. 3 shows the percentage

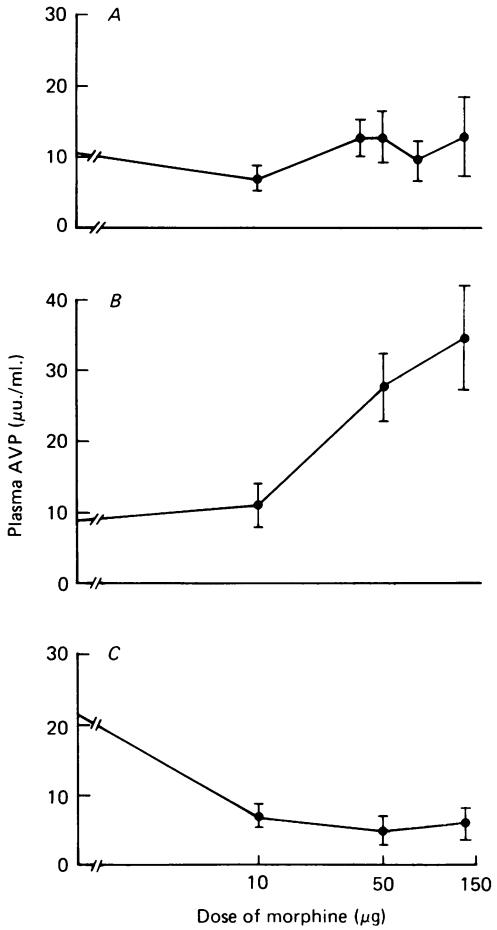

Fig. 2. Log dose–response curves for the vasopressin response to I.C.V. morphine at (*A*), 1, (*B*), 10 and (*C*), 30 min after the injection. The values represent the mean of six observations ± S.E.

change in mean arterial blood pressure produced by the various doses. Injections of saline and naloxone alone had no effect on blood pressure. The blood pressure increase commenced within 1·5 min of the morphine micro-injection, peak values were reached in 1·5–8 min and the duration of response varied from 7 to 15 min. Naloxone (0·5 mg/kg) completely abolished the increase of 17·2 ± 4 % observed with injection of 10 μg morphine. However, the response (28 ± 10 % increase in blood pressure) to injection of 50 μg morphine was only halved by this dose of naloxone. The observation that the time course of blood pressure changes differed from changes in plasma AVP

concentration, and the fact that naloxone reversed vasopressin response to morphine but did not completely inhibit the blood pressure changes, suggest that the blood pressure and vasopressin responses to morphine are independent of each other and may involve the activation of different opiate receptors.

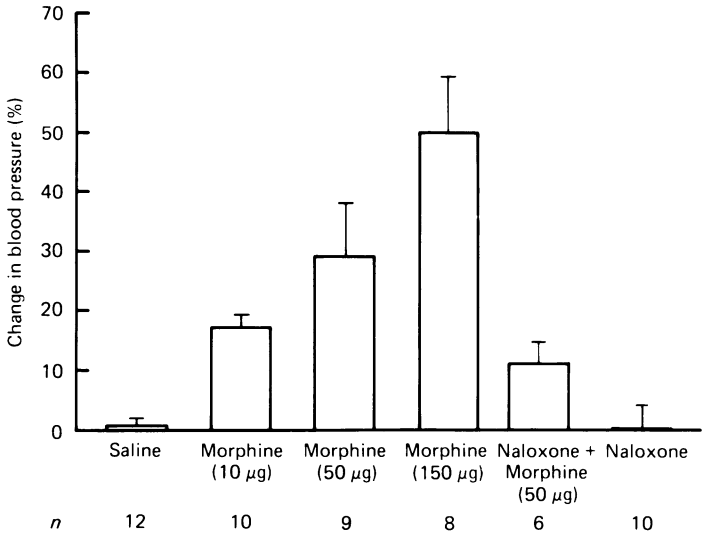

Fig. 3. The effect of I.C.V. morphine on blood pressure in the presence and absence of naloxone. The number of observations (n) is indicated.

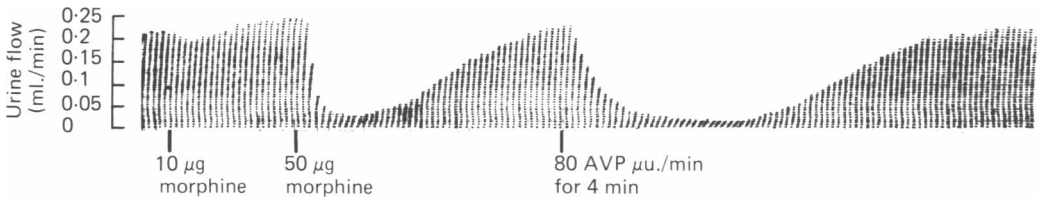

Fig. 4. The effect of I.C.V. morphine and intravenous infusion of vasopressin on urine flow in the anaesthetized, water-loaded rat.

In the two rats given I.C.V. injection of 150 μg (+) morphine, an elevation of blood pressure was not seen. On the contrary a hypotensive effect was observed, indicating that the blood pressure response to morphine is also stereospecific.

No correlation was found between the initial plasma AVP concentration and initial blood pressure, measured prior to withdrawal of the first blood sample in the rats used in this study.

The effect of I.C.V. micro-injections of morphine on urine outflow

Injections of both 10 and 50 μg morphine produced an immediate antidiuresis. Following the injection of 10 μg an antidiuresis of 12·5–46 % was seen with a mean of 11·5 min duration. After 50 μg morphine the antidiuresis was more pronounced,

being 18·4–79% and lasting a mean of 30·3 min. The response to 10 μg could be approximately matched by an infusion of vasopressin at 60 μu./min for 1 min and that to 50 μg by an infusion of 60–80 μu./min for 4–8 min. An example of the response is shown in Fig. 4.

The flow returned to pre-injection levels in all cases.

DISCUSSION

Recent studies by various groups have established that the effect of morphine on vasopressin release may not be a straightforward stimulation as proposed by de Bodo (1944). The response is largely dependent on the route of administration of morphine. Systemic administration results in a fall of blood pressure which could reflexly stimulate vasopressin release (Bisset, Black, Hilton, Jones, Kanjanapothi & Montgomery, 1974). Morphine has also been shown to have a direct effect on the kidney (Huidobro & Huidobro-Toro, 1979) which could be significant in studies were urine flow is taken as an index of the neurohypophysial response. Central administration of morphine also has an effect on the cardiovascular system, but in this instance it is a rise which would tend to inhibit vasopressin release. The present studies also revealed that centrally administered vasopressin has a biphasic effect on vasopressin release, namely a short-latency stimulation and a long-latency inhibition.

Morphine given intravenously or into the cerebral ventricles has clearly contrasting effects on the cardiovascular system. In the present studies a dose-dependent increase in blood pressure was seen over the whole range studied. A number of other groups have reported on the effect of centrally administered opioids on blood pressure. Schaz, Simon, Rockhold, Unger & Ganten (1979) described a dose-dependent increase in systolic arterial blood pressure and heart rate with I.C.V. infusion of leucine-enkephalin in spontaneously hypertensive and normo-tensive rats. Feldberg & Wei (1978*a*, *b*) observed a short-lasting rise in arterial blood pressure after intracerebroventricular injections of 400 μg morphine in the chloralose-anaesthetized cat which resulted from sympathetic stimulation and an action on structures in the wall of the third ventricle.

In the present study the response to vasopressin was both dose- and time-dependent. The only effect seen with injection of the lowest dose of 10 μg was an inhibition of vasopressin release of long latency. The higher doses of 50 and 150 μg morphine produced an initial stimulation of vasopressin release followed by an inhibition at 20 and 30 min.

The nature of the vasopressin response to morphine could well depend on the background activity of the neurohypophysial system. Thus in the present studies the inhibition of vasopressin release represents a reduction of circulating concentrations which had been elevated by anaesthesia and surgery. Similarly van Wimersma Greidanus *et al.* (1979) only reported morphine-induced inhibition of vasopressin secretion when concentrations were initially elevated as a result of saline treatment or water deprivation. In contrast, in the studies of Bisset *et al.* (1978) the basal levels of vasopressin were low as a result of water loading and the administration of alcohol anaesthesia. The same is true of the present studies when the antidiuretic response was monitored. Urethane was used as the anaesthetic but the vasopressin concentrations were still low in response to water loading. In this preparation I.C.V. injections

of morphine in doses of both 10 μg and 50 μg produce an immediate antidiuretic response. No enhanced diuresis was seen in the long term, probably because of the already reduced neurohypophysial activity.

Thus the seemingly contradictory results could be explained in terms of the species, the route of administration of morphine, the background activity of the neurohypophysial system, the dose employed and a time factor. Another possibility has been raised by Huidobro & Huidobro-Toro (1979). They observed that the antidiuresis following an I.C.V. injection of morphine could not be matched by vasopressin injections, the pattern of solute excretion being quite different in the two cases. The fact that 10 μg morphine produced no immediate change in measured plasma vasopressin concentrations while the same dose produced an antidiuresis in the water-loaded animals could point to the same conclusion. The present study however was not designed to answer this question, as neither the free water clearance, parameters of renal haemodynamics nor the electrolyte excretion were studied.

The site at which morphine could be acting is also a matter of some speculation. It could be on some pathway influencing vasopressin release or on the neurohypophysial system, either at the site of release in the posterior pituitary or in the hypothalamus. An enkephalinergic pathway from the hypothalamus to the posterior pituitary has been described by Rossier *et al.* (1979) and it is possible that morphine influences vasopressin release by activating receptor sites along this pathway. Different type of opiate receptors are present in the hypothalamus (Chang, Cooper, Hazum & Cuatrecasas (1979). Several groups have reported on the effects of endogenous opioids on vasopressin release. Van Wimersma Greidanus *et al.* (1979) observed decreased plasma AVP levels in rats subjected to I.C.V. injection of β-endorphin. However, Weitzman, Fisher, Minick, Ling & Guillemin (1977) reported a rise in plasma AVP in the conscious rabbit following intravenous administration of β-endorphin. In a more recent study Firemark & Weitzman (1979) observed a biphasic stimulatory response following on I.C.V. injections of β-endorphin and morphine.

In contrast Clarke, Lincoln & Wood (1980) observed an inhibition of firing of discharging vasopressinergic neurones following I.C.V injections of morphine in the anaesthetized rat. In an earlier report Clarke, Wood, Merrick & Lincoln (1979) observed an inhibition of oxytocin release at the level of the neurohypophysis produced by I.C.V. morphine, supporting the work of Haldar & Sawyer (1978) on the inhibition of suckling-induced oxytocin release in mice by subcutaneous injection of morphine.

Several investigations have also been carried out to determine if morphine affects AVP release at the level of the posterior pituitary. In 1977 Weitzman *et al.* found no effect of naloxone or β-endorphin on isolated rat posterior pituitary lobes. Iversen, Iversen & Bloom (1980) have reported an inhibition of stimulus-evoked release of vasopressin after addition of 10 μM-morphine to the isolated rat posterior pituitary. It is possible that the long-latency inhibition of vasopressin released we observed with various doses of morphine is due to a direct action of morphine at the level of the posterior pituitary.

In summary these results suggest that opiate receptors may play a role in the control of vasopressin secretion. Since there are opioid receptors in the hypothalamus, these observations could be of some physiological significance.

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