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Release of Antidiuretic Hormone by Morphine in Rats: An *in Vivo* and *in Vitro* Study[1] (41318)

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Abstract. Morphine has been reported to cause either diuresis or antidiuresis in rats. It is not clear whether antidiuretic hormone (ADH) release contributes to antidiuresis. In this paper we report that iv injection of morphine in doses of 0.5, 1, and 2 mg/kg caused antidiuresis and not diuresis in conscious rats. There was a dose-related increase in plasma ADH levels. The peak level of hormone occurred in the sample taken within 30 sec of morphine administration. The levels of hormone gradually decreased in samples taken at 5, 10, and 15 min after morphine administration. Prior injection of naloxone, 1 mg/kg, did not prevent the release of ADH in response to morphine. In *in vitro* experiments morphine did not release ADH into the medium when incubated with isolated neural lobes although a gradual release was observed when incubated with intact hypothalamoneurohypophysial preparations. The *in vitro* ADH release by morphine was also not prevented by naloxone. These results thus demonstrate that morphine can release ADH *in vivo* and *in vitro* but this action is not blocked by naloxone.

The finding of de Bodo (1) that morphine caused antidiuresis in dogs and that neurohypophysectomy blocked this response, suggested that morphine caused antidiuretic hormone (ADH) release. Although this observation was repeated by several other investigators (2–4) this concept, originally proposed by de Bodo, has been challenged. There are reports suggesting that morphine-induced antidiuresis is solely (5), or at least partly (6), due to changes in renal hemodynamics. It has even been reported that morphine can produce diuresis rather than antidiuresis but only in rats which were not water loaded during experimentation (7). Furthermore, the findings of Mills and Wang (8) even suggest that morphine might act as an inhibitor of ADH release since they observed that morphine blocks the antidiuretic response to afferent ulnar nerve stimulation in water-loaded dogs.

In most of these earlier studies antidiuresis was taken as indication that morphine released ADH. Direct measurement of ADH in plasma is necessary for obtaining unequivocal evidence. However, both the earlier reports using unextracted plasma and bioassay (9, 10) and recent reports using extracted plasma and specific radioimmunoassay (11–13) apparently came to different conclusions. On one hand, morphine injection into the cerebral ventricles caused ADH release in rats (11) and rabbits (12). On the other hand, van Wimersma Greidanus *et al.* (13) found that similar injection into rats inhibited ADH release. Thus the effect of morphine on ADH release is unclear and further investigations appear needed.

To investigate the issue three different types of experiments were performed. First, the effect of morphine on urine output in both hydrated and nonhydrated rats was tested. Next, hormone levels in plasma of conscious rats following iv morphine administration was estimated and, finally, *in vitro* incubation experiments were performed with both isolated neural lobes and the intact hypothalamoneurohypophysial system (HNS) to determine the site of action of morphine.

Materials and Methods. *Experiments in vivo. Cannulation.* All experiments were

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performed on female Sprague–Dawley rats weighing between 200 and 220 g. Rats were anesthetized with ip sodium pentobarbital, 45 mg/kg, during surgery. A polyethyline cannula (PE 50) was inserted into the jugular vein. For the experiments in which hormone was estimated in blood a carotid artery was also cannulated (PE 50). Both cannulae were passed under the skin and brought out between the ears. Cannulae were stored coiled in small Plexiglas cap (1-cm height and 2-cm diameter) sutured to the skin. The arterial cannula was filled with heparin (1000 U/ml, Wyeth Co.) and the venous cannula was filled with 0.9% saline.

Measurement of urine output. Before these experiments rats were deprived of food for 16 hr, but were allowed free access to water before and during experiments. Experiments were conducted either with water-loaded rats which received warm tap water by stomach tube (5% of their body weight at 0 hr) or with nonhydrated rats. During experiments rats were placed in individual metabolic cages and were injected with 20 μl of distilled water or with morphine solution (Merck) through the venous catheter which was implanted on the previous day. Urine volumes were noted for 6 hr. No effort was made to empty the rats bladder before measuring the volume of urine.

Estimation of ADH in plasma. Experiments were performed on the days following cannulation. Rats were injected either with distilled water or with morphine through the venous cannula and 2 ml of blood was collected from the arterial cannula by means of a plastic syringe containing 0.2 ml of heparin (50 U/ml). An equal volume of dextran (6% in isotonic saline, Abbott Laboratories) was injected simultaneously through the venous cannula. Following morphine injection, blood samples were collected either at 15–30 sec (for dose–response curve experiments) or 5, 10, and 15 min (for release pattern experiments). Plasma obtained from these samples by 20 min centrifugation at 3000 rpm at 4° was extracted immediately following the method of Husain *et al.* (14). Extracted samples were kept in the freezer and were assayed within 3 days. ADH levels in these extracted samples were determined by vasopressin RIA.

Blood pressure recording in conscious rats. For these experiments jugular and carotid artery cannulae were inserted on the previous day as described earlier. In some later experiments cannulae were put in the femoral vein and femoral artery instead. The artery cannula was connected to a pressure transducer (Statham, P233GC) and recorder (Grass, Model 79). When steady blood pressure recordings were obtained rats were injected iv with morphine.

Experiments in vitro. Incubation of isolated neural lobes. All experiments were performed on female Sprague–Dawley rats weighing between 175 and 200 g. These were decapitated, the pituitary gland was removed, and the neural lobe was isolated. The neural lobe was cut along the sagittal plane and the two halves were then transferred to the incubation tube (10 × 75 mm). Each neural lobe was then incubated in 1 ml modified Locke's solution (15) in a Dubnoff metabolic shaker at 37° and gassed with 95% O_2 and 5% CO_2. The incubation medium had the following composition (m*M*): NaCl 154; KCl 5.6; $CaCl_2$ 2.2; $MgCl_2$ 1.1; glucose 10; and $NaHCO_3$ 6. With any change in potassium concentration, NaCl concentration was adjusted so that the total osmolality stayed the same. The medium was removed every 10 min and was replaced with fresh medium. The first two incubation samples were discarded, the third, fourth, and fifth samples served as precontrol, test, and postcontrol, respectively. Samples thus collected were kept in the freezer until needed for RIA. Assays were performed within a week. All results are expressed as the amount of hormone released per milliliter per 10 min.

Incubation of hypothalamoneurohypophysial system (HNS). Isolation of HNS was as follows. Following decapitation the brain was removed using a caudal approach. The sella was removed and the pituitary and stalk were exposed. The anterior pituitary was removed and discarded. The dura mater and arachnoid were carefully pulled away. A block of tissue was then isolated by cutting rostral to the optic chi-

asm, lateral to the median eminence to a depth of 4 mm. Thus this preparation contained intact supraoptic nuclei (SON), paraventricular nuclei (PVN), hypophysial stalk, and neural lobe. The entire preparation weighed 120 ± 4.4 mg (mean ± SE, $n = 10$).

The HNS preparation thus obtained was transferred to an incubation tube (10 × 75 mm) containing 1 ml medium (16). The medium contained (mM): NaCl 126; KCl 6; Na_2HPO_4 1; $MgSO_4$ 0.88; $NaHCO_3$ 22; $CaCl_2$ 1.45; and glucose 0.2%. The medium was gassed with 95% O_2 and 5% CO_2 and had a pH of 7.4. The tissue was incubated at 37° in a Dubnoff metabolic shaker. After an equilibrium period of 30 min the tissue was incubated up to 120 min during which the medium was changed in the following sequences, 30–60 min, 60–70 min, 70–90 min, and 90–120 min to determine the basal hormone release pattern. Stimuli were always applied during the 60- to 70-min period and the 70- to 90-min incubation period was used as postdrug period. The fourth sample, i.e., the 90- to 120-min sample, was taken only during the determination of basal hormone release rate. Samples were kept in the freezer until RIA assay was performed which was done within a week. All results are expressed as the amount of hormone released per milliliter per 10 min. Results are standardized by expressing postdrug release rates as a percentage of the predrug control release rates.

Extraction. Plasma samples were extracted with cold acetone essentially following the method of Husain *et al.* (14). In summary, plasma proteins were separated with acetone and plasma lipids were separated with petroleum ether. Samples were then evaporated under a stream of air and kept in cold and were reconstituted with RIA buffer for assay. Recovery of a known amount of vasopressin performed with Brattleboro rat plasma was 65 ± 9% (mean ± SE, $n = 10$).

Radioimmunoassay. Iodination. Five micrograms of synthetic arginine vasopressin (a gift from Dr. M. Manning with a potency of 340 ± 9 U/mg assayed against USP standard) were iodinated by the lactoperoxidase method of Thorell and Johansson (17). The iodination mixture was transferred to a glass tube containing 500 mg of anion exchange resin (AG 2 × 8 in acetate form, Bio-Rad Co.) to absorb the remaining unreacted iodine. The iodide–resin mixture was stirred with a Vortex (Vortex-Genie) shaker for 5 min and then was centrifuged at 3000 rpm for 15 min. The supernatant solution was applied to a 1 × 20-cm column of Sephadex G-25 (Pharmacia) and eluted with 0.2 *M* acetic acid containing 1.25% bovine serum albumin. Two peaks of radioactivity were routinely obtained and a fraction of the descending limb of the second peak was used for RIA.

Antibodies. Determination of ADH in plasma samples was carried out using Glick-1 antiserum (14). This antiserum was obtained as a gift from Dr. R. Stark and from Dr. K. Husain. Since the supply of Glick-1 antiserum was limited, determination of ADH in incubation samples was carried out with R-70 antiserum (18). This antiserum was a gift to Dr. W. H. Sawyer from Dr. A. A. Rosenbloom and Dr. D. A. Fisher.

Radioimmunoassay procedures. The same method was applied for RIA whether Glick-1 antiserum or R-70 was used. Standard curves were prepared in triplicate using 0.1–10 pg AVP for Glick-1 antiserum and 0.78–100 pg for R-70 antiserum. The diluent buffer used in these experiments was 0.05 *M* sodium phosphate, pH 7.5, containing 10 m*M* EDTA, 150 m*M* NaCl, 0.1% sodium azide, and 0.25% normal rabbit serum. The total volume of the incubation samples was 500 μl (200 μl buffer, 50 μl antiserum, 50 μl ^{125}I-AVP solution, and 200 μl standard or unknown solution). Samples were incubated with the antiserum for 24 hr at 4° before addition of ^{125}I-AVP and then for an additional 24 hr after addition of ^{125}I-AVP. Separation of bound from free ^{125}I-AVP was carried out by addition of 50 μl of bovine γ globulin and 0.5 ml of a 25% aqueous solution of polyethelene glycol (Carbowax 6000) to each tube, thoroughly mixing them, and centrifuging at 4000 rpm for 30 min. The samples were counted on a Searle 1195 automatic gamma counter. The ratio of bound (B) to total (T) ^{125}I (B/T) obtained in the absence of unlabeled hormone was usually 60–70%. Bound counts were

not corrected for nonspecific precipitation of ^{125}I (in the absence of antiserum). The nonspecific precipitation was consistently less than 6%. Both Glick-1 and R-70 antisera were used in a final dilution of 1:100,000. The sensitivity of the assay with Glick-1 antisera was 0.1 pg/tube while with R-70 it was 1 pg/tube. Between assay variation (a sample containing 10 pg/tube was assayed in five different assays) was 15.2% and within assay variation (samples containing 5 pg/tube was assayed 20 times in one assay) was 5.1%. Values given for plasma AVP have been corrected for losses during extraction. Incubation medium up to 200 μl with or without 200 ng of morphine did not cause any interference when added together with the standard. Known amount of AVP added to the incubation medium produced 93 ± 8% (SD, n = 12) recovery.

Correlation between RIA and bioassay. Two plasma samples and four incubation samples were tested both by RIA and by bioassay. For determination of bioassayable activity antidiuretic assays were performed by the method of Sawyer (19). For each sample at least two 4-point assay groups were carried out. Incubation samples were selected at random from neural lobe incubation experiments. Of the two plasma samples one was a single sample and the other was a pooled sample from three experiments. There was a close agreement between values obtained by two assay procedures (r = 0.97) with a slope of 0.77 and an intercept of 0.18.

For all experiments, both *in vivo* and *in vitro,* the significance of drug effects on the release was evaluated using the unpaired Student's t-test. Also, values given for experiments represent mean ± SE.

Results. *Effect of morphine on urine output: hydrated and nonhydrated rats.* An iv injection of morphine in a dose of 0.5 mg and 1 mg/kg into hydrated rats caused antidiuresis (Fig. 1A). With the smaller dose, i.e., 0.5 mg/kg, there was a significant difference ($P < 0.02$) from control at 1 hr. In later hours, the differences were not significant, e.g., at 4 hr $P < 0.19$. However, with 1 mg/kg dose, the urine output was significantly lower ($P < 0.01$) even at 6 hr. More interesting, however, was the finding

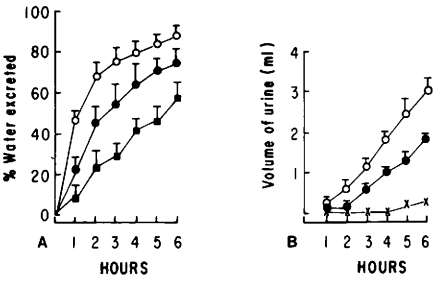

FIG. 1. The effect of morphine on urine output of hydrated (A) and nonhydrated (B) rats. For hydrated rats results are expressed as percentage of water excreted at every hour up to 6 hr with control (○), 0.5 mg/kg (●), and 1 mg/kg (■) morphine. The percentage of water excreted was defined as the volume of urine excreted divided by the volume of fluid load multiplied by 100. With nonhydrated rats the results are expressed on actual urine output at every hour for 6 hr for control (○), 0.5 mg/kg (●), and 1 mg/kg (x) of morphine. Values given for all the experiments are the mean ± SE, n = 6.

that even in nonhydrated rats these two doses of morphine caused profound antidiuresis which persisted up to 6 hr (Fig. 1B). Furthermore, in nonhydrated rats urine output was completely shut down for 2 hr with the small dose (0.5 mg/kg) and for 4 hr with the higher dose (1 mg/kg). Thus even in nonhydrated rats no diuresis was noticed after morphine.

Control plasma ADH level. Several experiments were performed initially in which control blood samples were collected from rats following decapitation. In spite of gentle handling, the plasma ADH levels in these rats were high, i.e., 10 ± 2 pg/ml (n = 20). This problem was circumvented by collecting blood samples from precannulated carotid arteries and by injecting an equal volume of dextran simultaneously. Using this procedure low plasma ADH levels, i.e., 3.6 ± 0.1 pg/ml (n = 9), were obtained; however, even with this method it was necessary to take blood very fast since it was experienced that slow collection of blood would raise plasma AVP levels. Thus whenever it took more than 30 sec to collect 2 ml of blood the sample was discarded. This rule was followed both for control and for morphine experiments.

Effect of morphine on plasma ADH level.

Figure 2A shows that morphine in doses of 0.5, 1, and 2 mg/kg iv caused a dose-related rise in plasma ADH levels. Rises due to all these three doses were highly significant ($P < 0.002$). There was an excellent correlation between plasma ADH level and morphine dose as is described in the equation VP = 0.218 + 23.9 (morphine), $r = 0.98$, ($p < 0.01$).

In the next series of experiments the time course of release of ADH by morphine was determined. In these experiments morphine was injected in a dose of 1 mg/kg iv and blood samples were collected at 15–30 sec and at 5, 10, and 15 min. As can be seen in Fig. 2B the maximum release, 19.5 ± 1.05 pg/ml ($n = 5$), was observed in the 15- to 30-sec sample and the level gradually decreased in the later samples. The ADH level in the 5-min sample was 12.5 ± 0.86 pg/ml ($n = 4$) which was significantly lower ($P < 0.001$) than the first sample (15–30 sec) and the level in the 10-min sample, 7.5 ± 0.64 pg/ml ($n = 4$), was significantly lower ($P < 0.002$) than the 5-min sample. The ADH level in the 15-min sample was lower than the 10-min sample but higher than control although it was not significantly different ($P < 0.44$) from the control.

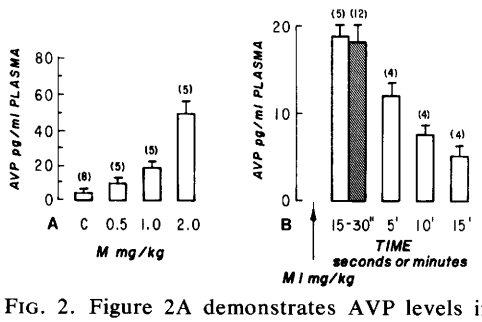

FIG. 2. Figure 2A demonstrates AVP levels in plasma (pg/ml) in control and following 0.5, 1, and 2 mg/kg doses of iv morphine (M) administration. All blood samples were collected within 1 min following morphine injection. Figure 2B demonstrates the pattern of AVP release (open columns) following 1 mg/kg dose of morphine on samples taken at 15–30 sec and at 5, 10, and 15 min. The hatched column represents the amount of hormone released in 15- to 30-sec sample taken from rats which received iv naloxone (1 mg/kg) 10 min prior to the injection of morphine. All values given are the mean ± SE. Number given in parentheses represents n for that group.

Since the highest level of ADH was observed in the 15- to 30-sec sample the effect of naloxone on morphine-induced ADH release was determined on samples taken at this time only. Injection of naloxone in a dose of 1 mg/kg iv 10 min prior to the injection of morphine did not reduce ADH release. The hormone level was 18.8 ± 1.25 pg/ml ($n = 12$). Given alone, naloxone in a dose of 1 mg/kg iv had no effect on the ADH level in the 15- to 30-sec sample (4.2 ± 0.3 pg/ml, $n = 5$).

Effect of morphine on blood pressure of conscious rats. The average mean blood pressure of these conscious rats was 125 ± 3.8 mm Hg (SE, $n = 33$). Injection of morphine into these rats always caused an immediate sharp fall of blood pressure. After 0.5 mg/kg dose the fall was 48.7 ± mm Hg ($n = 15$) and after 1 mg/kg the fall was 63.75 ± 6.25 mm Hg ($n = 6$). Naloxone in a dose of 1 mg/kg injected iv 10 min prior to the injection of 1 mg/kg of morphine completely blocked the hypotensive effect of morphine. The mean arterial pressure for the group which received both naloxone and morphine was 128.3 ± 3.3 mm Hg ($n = 6$) and in those which received only naloxone was 131.6 ± 4.2 mm Hg ($n = 6$).

Incubation experiments. Basal hormone release rate was determined from 6 neural lobe experiments and from 19 HNS experiments. For neural lobe the release rate in third, fourth, fifth, and sixth samples was 7.7 ± 0.70, 5.6 ± 0.93, 4.5 ± 0.86, and 4.7 ± 0.43 ng/ml/10 min, respectively. For HNS, the basal release rate in second, third, fourth, and fifth samples was 319 ± 20, 200 ± 17, 193 ± 20, and 80 ± 7 pg/ml/10 min, respectively. In neural lobe preparation the application of 56 mM KCl caused an almost 10-fold increase in ADH release, 40 ± 1.5 ng/ml/10 min ($n = 6$).

Figure 3 shows the effect of morphine on isolated neural lobes as well as on HNS preparation. Morphine in a concentration of 1.4×10^{-10}–1.4 ± 10^{-7} M did not cause any release of ADH when incubated with isolated neural lobes but a graded release was obtained when morphine was incubated with HNS. With the smallest dose, i.e., 1.4×10^{-10} M (A), there was a 140 ± 18% ($n = 4$) increase over the control ($P < 0.05$).

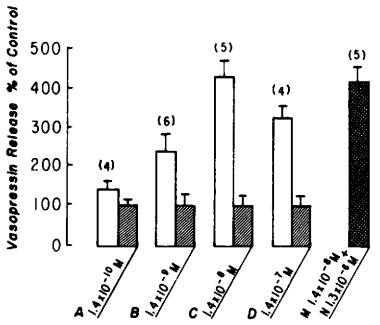

FIG. 3. Effect of morphine (M) in doses 1.4×10^{-10}–1.4×10^{-7} *M* on AVP release from the HNS (open columns) and neural lobes (hatched columns). Results were standardized by expressing postdrug release values as a percentage of the predrug control release values. Number given in parentheses represents *n* for that group. All neural lobe experiments $n = 6$.

With higher doses, i.e., 1.4×10^{-9} *M* (B) and 1.4×10^{-8} *M* (C), the increases were 248 ± 18% ($n = 6$) and 430 ± 44% ($n = 5$), respectively. The rate of release was also significantly more ($P < 0.002$) for B than A and for C than B. With the highest concentration, i.e., 1.4×10^{-7} *M* (D), no further stimulation was noticed; instead the ADH level in this incubation sample was lower, but this lower level was not significantly different ($P < 0.06$) from that obtained by dose C. In all these experiments ADH level in the 70- to 90-min sample was lower than the previous sample suggesting that the stimulatory effect of morphine did not persist after the drug was removed. Release of ADH by 1.4×10^{-8} *M* morphine was not blocked by naloxone added 1 min prior to the addition of morphine even when naloxone was added at a concentration of 1.3 ± 10^{-6} *M* ($n = 5$).

Discussion. The results presented in this paper clearly demonstrate that injection of morphine into rats causes antidiuresis as well as release of ADH into the blood. The urine output in hydrated rats confirms all the previous reports (2–4) but the results obtained with nonhydrated rats do not agree with those reported by Marchand (7). It is difficult to explain why these results were different from those of Marchand although it could be due to different routes of injections since in the previous study (7) morphine was injected sc while in the present investigation it was injected iv. The rapidity of rise in plasma morphine arriving at the hypothalamus after iv administration, compared to sc, could make some difference. Recently Iversen *et al.* (20) have demonstrated that the release of ADH from the isolated posterior pituitary by electrical stimulation of the stalk was inhibited by morphine. Thus there may be both stimulation at the hypothalamic level and inhibition at the neural lobe level and the pattern of rising morphine concentration may determine the overall response. In any event, since antidiuresis was observed both in hydrated and nonhydrated experiments ADH levels in plasma was measured following morphine administration.

Results presented in this paper demonstrate that iv administration of morphine consistently raised ADH levels and that this response was dose related. These results are contradictory to those reported by van Wimersma Greidanus *et al.* (13) who demonstrated that morphine inhibits ADH release from stimulated and nonstimulated pituitary glands of rats *in vivo*. It is difficult to make a fair comparison between the results obtained in these two investigations since the dose of morphine used by van Wimersma Greidanus *et al.* (13) is extremely high, 30 mg/kg compared to 1 mg/kg used in the present investigation. From the experiments on pattern of release it was apparent that the release of hormone occurs immediately after injection. It is difficult to say whether the amount of ADH detected in the later samples was the remainder of hormone released initially or reflected continued release by morphine, since reported half-lives for ADH in rats are highly variable (cf. (21)). Spector (22) has demonstrated that in rats elimination of morphine occurs in a biphasic manner; for the first phase the half-life for morphine is 50 min and for the second phase the half-life is 6.8 hr. Thus it might be possible that the effect of morphine would continue for a while. Firemark and Weitzman (12) in their experiments on rabbits reported maximum release of ADH in the first 60 min following morphine injection. Since they did not estimate hormone levels in blood immediately after injection it cannot be said whether re-

lease was maximal in the first minutes as in the present experiments. However, since these investigators used an intraventricular route for injection, while we used iv, peak level of hormone could naturally occur at different times.

The *in vitro* incubation experiments with isolated neural lobes and with HNS indicated the possible site of action of morphine in causing ADH release. The fact that the high KCl solution (56 m*M*) caused severalfold increase in ADH release from isolated neural lobes whereas morphine was ineffective strongly suggests that the neural lobe most likely is not the site where morphine acts to release ADH. Application of morphine to the HNS, in which the neural lobe remains attached to the hypothalamic nuclei, effectively released ADH. In the *in vitro* experiments the dose of morphine which elicited release of ADH was several orders of magnitude less when compared with that of the *in vivo* experiments. Assuming the blood volume in rats in 67 ml/kg the plasma concentration of morphine would be 14.92 μg/ml following 1 mg/kg dose. In the *in vitro* experiments the highest release of ADH was achieved with 1.4×10^{-8} *M* which is equivalent to 10.6 ng/ml. It is obvious that although this calculation of plasma morphine concentration is correct theoretically, most likely this does not represent the concentration of morphine at the receptor level and it is the receptor concentration which should be taken into account for its action. However, the finding that in a similar setup the same concentration of morphine did not release ADH from the neural lobe whereas it caused dose-related release from the HNS would strongly suggest that morphine acts somewhere higher in the system than the neural lobe. Recently Iversen *et al.* (20) demonstrated that morphine acts at the neural lobe to cause inhibition of vasopressin release induced by stalk stimulation. The apparent conflict between this finding and those reported above could well be due to one of the following. First, for any agent the site and/or sites for stimulatory and inhibitory action could be different and second, the action of morphine on unstimulated neural lobes (the condition used in the present experiments) and stimulated neural lobes (the conditions used in Iversen's experiments) could be different. For example, agents such as β-endorphin (23), cAMP, theophylline (24) acted differently when tested in *in vitro* neural lobe preparation depending on whether the neural lobe was stimulated or unstimulated. We also do not know whether morphine acts directly on the hypothalamic nuclei themselves, thereby causing ADH release, or whether morphine releases some other agent such as acetylcholine which in turn releases ADH from the neural lobe. Bridges *et al.* (16) have demonstrated that hypothalamic nuclei alone are capable of releasing both hormones, OT and VP, when stimulated by appropriate stimuli such as acetylcholine or dopamine.

Ginsburg and Brown (25) reported that in rat a 50-mm Hg fall of BP would be sufficient to trigger ADH release. Although in the present experiments the fall of BP due to morphine was similar, i.e., 48.7 ± 4.76 ($n = 15$) with 0.5 mg/kg and 63.7 ± 6.25 ($n = 6$) with 1 mg/kg, it is not the fall alone which caused ADH release since naloxone, 1 mg/kg dose, completely blocked the hypotensive effect of morphine while the same dose did not block the increase in ADH release in plasma following morphine administration. Kanjanapothi (26) also demonstrated that morphine could cause antidiuresis and increased excretion of ADH in rats without producing hypotension.

In the present series of experiments the morphine-induced increase in plasma ADH levels was not altered by prior injection of naloxone. Similarly, release of ADH from HNS by morphine was not inhibited by the presence of naloxone in the medium. Weitzman *et al.* (27) have also shown that naloxone does not inhibit spontaneous ADH release from isolated posterior pituitary lobe. Recently Firemark and Weitzman (12) reported that morphine-induced ADH release in rabbits could not be reversed by naloxone. Thus the type of receptor that morphine acts on to cause ADH release appears unresponsive to naloxone. It is fairly well established now that there are multiple types of opiate re-

ceptors (28, 29). Indeed there are several reports that some other actions of morphine are not antagonized by naloxone (30–32).

Although it seems clear that morphine can release ADH this may not be the only mechanism by which it influences renal function. For example, a recent report of Huidoboro and Huidoboro-Toro (33) demonstrated that, although both morphine and AVP caused antidiuresis in rats, the excretion of electrolytes in the urine following injection of these two agents is different.

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