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EFFECTS OF MORPHINE AND β-ENDORPHIN ON BASAL AND ELEVATED PLASMA LEVELS OF α-MSH AND VASOPRESSIN

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Summary

Morphine induced an increase of plasma α-MSH levels and a decrease of AVP levels after peripheral or intracerebroventricular administration. This increase of α-MSH levels and decrease of AVP levels after morphine treatment was observed in non-stimulated animals as well as in rats in which the hormone levels were elevated by water deprivation or by administration of hypertonic saline. These latter effects of morphine on plasma levels of α-MSH and AVP could be blocked by simultaneous administration of naltrexone.

β-Endorphin also increased plasma α-MSH levels and lowered plasma AVP levels. From these effects only the increase of the plasma α-MSH level and not the decrease of plasma AVP could be blocked by naltrexone. Moreover PLG treatment was ineffective with respect to the endorphin-induced decrease in plasma AVP, but it partly blocked the increase of plasma α-MSH when this tripeptide was given in combination with β-endorphin.

It has been well established that morphine stimulates or inhibits the release of various pituitary hormones. De Wied et al. (1), Kokka et al. (2) and Martin et al. (3) reported an increased release of GH following morphine treatment in rats and Lal and coworkers (4) demonstrated that morphine induces an increase in prolactin release. Similarly, morphine stimulates GH and prolactin release in goats and this effect was completely blocked by the morphine antagonist naloxone (5). Moreover both stimulation and inhibition of ACTH release by acute morphine treatment has been observed in various species by several authors (2,6-10). The release of LH and of FSH is reduced by morphine and this drug significantly decreased serum TSH concentrations (11).

In addition morphine administration has been reported to stimulate the release of hormones from the neuro-intermediate lobe. MSH is released after morphine (12) and it has been suggested that both morphine and peptides with opiate like activity will stimulate the release of vasopressin (13-17).

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In most of these studies, however, indirect parameters were used to measure hormone release by the pituitary gland or bioassays to determine plasma levels (16). Moreover the effects of morphine or opiate-like peptides on plasma levels of pituitary hormones were generally determined under basal or non-stimulated pituitary activity. In the present study the effects of morphine and of β-endorphin were studied on plasma levels of α-MSH and of vasopressin (AVP) by direct measurement of these hormones by specific radioimmunoassays under basal as well as under stimulated conditions.

Methods

Male rats of an inbred Wistar strain (TNO Zeist, the Netherlands), weighing 160-180 g were used. Morphine was injected intraperitoneally (ip) or intracerebroventricularly (icv). Icv injections were performed through a permanent polyethylene cannula, which had been placed under ether anesthesia into one of the lateral ventricles of the rat brain at least one week before experimentation. Animals were single housed and handled intensively before starting the experiment. Localization of the tip of the cannula was determined at the end of the experiment by icv injection of Evans Blue and macroscopical inspection of the staining of the rat's brain-ventricular system.

Determination of hormone levels was performed by specific radioimmunoassays (RIA) of α-MSH and of AVP using the same plasma samples, which were obtained after decapitation by collecting trunk blood in cooled heparinized plastic tubes. The RIA of AVP has been described in detail elsewhere (18). Iodination of AVP and achievement of the standard curve was performed with an extremely potent synthetic AVP preparation (Organon International b.v. Oss, the Netherlands, 509 IU pressor activity/mg). Sensitivity of the standard curve was 0.25 pg AVP/tube. Extraction of AVP from plasma was performed by use of activated Vycor glass powder (recovery ca. 65%). Plasma of homozygous diabetes insipidus rats was used for measuring recovery and for control of aspecific effects. Cross reactivity with vasotocin was 0.25% and with oxytocin < 0.1%. All results are corrected for recovery and expressed in pg/ml.

For the measurement of α-MSH a RIA was used as described by Penny and Thody (19) with slight modifications (20,21). Briefly the procedure is as follows. As diluent buffer a 0.05 M phosphate buffer PH 7.4 containing 0.2% human serum albumin and 0.05% mercaptoethanol was used. Ten µl ^{125}Iα-MSH (approximately 2000 cpm, specific activity ca. 320 µCi/µg) and 50 µl diluted rabbit antiserum to α-MSH (final dilution: 1:40.000, 50% initial binding) were added to 150 µl doubling dilutions of standard α-MSH or to appropriately diluted samples. Incubation time was 24 hrs at 4°C. Separation of free and bound tracer was achieved by charcoal precipitation. The sensitivity of the standard curve was 2 pg α-MSH/tube. No extraction from plasma was needed. Cross-reactivity was minimal with ACTH 1-10, ACTH 4-10, ACTH 4-7 and ACTH 7-10 (< .03%), with ACTH 1-24 (< .07%) and with ACTH 1-39 (0.5%), whereas ACTH 1-13 NH_2 had > 70% cross-reactivity, indicating that the antiserum is mainly directed towards the Lys-Pro-ValNH_2 C-terminal portion of the α-MSH molecule. All results are expressed in pg/ml.

In a first series of experiments morphine was injected ip (30 mg/kg) or icv (5 µg) and trunk blood was collected at different time intervals after morphine administration for measuring α-MSH and AVP content. Saline (0.5 ml) was used as placebo, for the ip injections, whereas for icv administration artificial CSF (2 µl) served as placebo.

In a second set of experiments the effects of morphine (icv, 1 µg) on plasma levels of α-MSH and AVP were determined 30 min after administration of the drug either in animals after 24 hr of water deprivation or in animals which had received a subcutaneous (sc) injection of 1.2 ml 15% NaCl solution containing 2.4% Procaine HCl, 3 hr before. In addition the effect of naltrexone (icv,1 µg) either alone or in combination with morphine on plasma levels of

Table 1a

Effect of ip injection of morphine (30 mg/kg) on plasma levels of α-MSH and of AVP after various time intervals

	5 min		15 min		30 min	
	pg α-MSH/ml	pg AVP/ml	pg α-MSH/ml	pg AVP/ml	pg α-MSH/ml	pg AVP/ml
Placebo	114.3± 8.2 (8)	1.6±0.4 (6)	118.0±14.0 (9)	1.6±0.4 (8)	120.2± 8.3 (9)	2.2±0.3 (9)
Morphine	142.8±17.8 (8)	0.8±0.2*(5)	185.3±12.3*(9)	0.7±0.2*(8)	207.4±21.0*(5)	1.0±0.3*(6)

* $p < .05$
( ) number of animals per group.

Table 1b

Effect of icv injection of morphine (5 μg/rat) on plasma levels of α-MSH and of AVP after various time intervals

	5 min		15 min		30 min	
	pg α-MSH/ml	pg AVP/ml	pg α-MSH/ml	pg AVP/ml	pg α-MSH/ml	pg AVP/ml
Placebo	75.6±7.3 (4)	2.0±0.4 (7)	81.8±3.0 (4)	2.5±0.6 (8)	83.4±7.0 (5)	2.9±0.8 (8)
Morphine	113.4±2.6*(4)	1.2±0.3 (7)	114.5±8.7*(3)	1.8±0.6 (8)	111.4±7.4*(4)	1.8±0.5 (8)

* $p < .05$
( ) number of animals per group.

α-MSH and of AVP was investigated in this series of experiments.

Finally the effect of β-endorphin (icv, 5 μg) on plasma levels of α-MSH and of AVP was examined as well as the effect of combined treatment of β-endorphin with naltrexone (icv, 1 μg) or with Pro-Leu-Gly (PLG, icv, 50 ng), which has been reported to have MSH-release inhibiting activity (21). Statistical analysis was performed by the students t-test or by the U test of Mann-Whitney (22).

Results

Plasma α-MSH levels were significantly increased over control values 15 min after ip injections of morphine and were still elevated at 30 min. In contrast, morphine decreased plasma AVP levels as compared with control values; this effect was detected 5 min after the drug and was still evident at 30 min (Table 1a).

Plasma α-MSH levels were significantly increased at 5, 15 and 30 min after icv administration of morphine. On the other hand, plasma AVP levels were decreased after icv administration of morphine, although this decrease did not reach statistical significance when morphine treated rats were compared with placebo's at the same time intervals. (Table 1b).

Table 2

Effects of morphine (icv 1 μg, 30 min prior to decapitation) and of naltrexone (icv 1 μg, 30 min prior to decapitation) on plasma levels of α-MSH and of AVP in rats after 24 hr water deprivation

	pg α-MSH/ml	pg AVP/ml
Placebo	167.8±4.3 (18)	12.7±0.9 (25)
Morphine	193.7±6.8*(16)	6.1±0.9*(21)
Naltrexone	175.8±7.2 (16)	12.8±0.8 (23)
Morphine + naltrexone	179.1±4.1 (18)	10.4±1.1 (16)

* $p < .05$
( ) number of animals per group.

Table 2 shows the results of icv injection of morphine and/or naltrexone on plasma α-MSH and AVP levels in rats submitted to 24 hr water deprivation. Under these conditions the administration of morphine resulted in an increase in plasma α-MSH levels. This effect was completely blocked by simultaneous administration of naltrexone. In contrast, morphine inhibited the stimulated AVP release and this effect of morphine was also blocked by simultaneous administration of naltrexone.

Morphine also increased plasma α-MSH levels in rats that had received a sc injection of 15% NaCl 3.5 h previously, but on the other hand decreased plasma AVP levels (Table 3). Both effects were blocked by naltrexone (Table 3). When administered alone naltrexone appeared to be opposite to morphine, in that plasma α-MSH levels tended to be lower and plasma AVP levels tended to be higher than corresponding control levels (Table 3).

Table 3

Effects of morphine (icv 1 µg, 30 min prior to decapitation) and of naltrexone (icv 1 µg, 30 min prior to decapitation) on plasma levels of α-MSH and of AVP in animals 3.5 hr after sc injection of 15% NaCl

	pg α-MSH/ml	pg AVP/ml
Placebo	145.4±11.6 (9)	29.3±2.0 (9)
Morphine	172.3± 8.1*(8)	12.2±2.0*(8)
Naltrexone	118.0± 9.2 (8)	35.3±3.4 (8)
Morphine + naltrexone	144.4±10.1 (8)	25.9±1.2 (8)

* $p < .05$
( ) number of animals per group.

Table 4

Effects of β-endorphin (icv 5 µg, 30 min prior to decapitation), naltrexone (icv 1 µg, 30 min prior to decapitation) and PLG (icv 50 ng, 30 min prior to decapitation) on plasma levels of α-MSH and of AVP

	pg α-MSH/ml	pg AVP/ml
Placebo	99.8± 5.1 (6)	2.7±0.4 (12)
PLG	100.1±14.1 (5)	3.1±0.7 (5)
β-endorphin	156.2±19.0*(5)	1.3±0.3*(11)
β-endorphin + naltrexone	104.0±11.7 (7)	1.2±0.2*(15)
β-endorphin + PLG	133.5±18.0 (8)	1.0±0.1* (8)

* $p < .05$
( ) number of animals per group.

Icv administration of β-endorphin produced a similar response as morphine with respect to plasma levels of α-MSH and AVP; i.e. an increase in α-MSH levels and a decrease in AVP levels (Table 4). The increase of plasma α-MSH levels after β-endorphin administration was completely blocked by simultaneous administration of naltrexone, whereas the decrease in plasma AVP levels after β-endorphin was not affected by simultaneous naltrexone administration (Table 4). Additionally, PLG was without effect on plasma α-MSH and AVP levels and had no effect on the inhibitory action of β-endorphin on plasma AVP, whereas the combination of β-endorphin and PLG resulted in a kind of intermediate response concerning plasma levels of α-MSH (Table 4).

Discussion

The present study clearly indicates that morphine affects plasma levels of the pituitary hormones α-MSH and AVP, probably by a stimulation of α-MSH release from the pituitary and an inhibition of AVP release from the neurohypophysis.

Morphine analogues have been shown to produce diuresis both in man (24) and rats (25) and it was concluded that these derivates inhibit the release of AVP. More recently, Haldar and Sawyer (26) have shown that morphine and its analogues will also inhibit the release of oxytocin. The present results therefore support these findings. Other workers, on the other hand, have reported an antidiuretic effect of morphine in rats and man and this effect, in rats but not in man was diminished by nalorphine (27). In addition intravenously administered β-endorphin was found to increase plasma AVP levels in rabbits but to have no effect on AVP release in isolated rat neural lobes (17). The possibility therefore exists that morphine and the opiate like peptides modify plasma AVP levels through an indirect effect. Morphine has been suggested to affect the release of pituitary hormones by interfering with neurotransmitter systems in the brain, particularly in the hypothalamus, and the hypothalamic catecholamines seem to be involved in this action of morphine (1,28). Interestingly, such a catecholamine involvement has recently been suggested with respect to the inhibitory effect of morphine on suckling induced oxytocin release in mice (26). These monoamines might therefore mediate the effect on AVP release. Another possibility is that morphine and the opiate peptides stimulate the excretion of AVP by the kidney, and this could result in a decrease in plasma AVP levels as seen in the present study, and the increase in urinary levels of antidiuretic activity after enkephalin reported by Bisset et al. (16).

The fact that Bisset et al. (16) found Leu-enkephalin and morphine to be more effective in stimulating AVP release when administered icv than by the iv route may be explained by the large volume of saline (10-25 μl) used as vehicle in their study. We have observed elevated levels of plasma AVP after icv injections of saline (2 μl) and we now therefore use artificial CSF as vehicle for injections into the ventricular system. A large volume of saline injected into the brain ventricular system may affect the osmoreceptor system in AVP release.

The present observation that ip administration of morphine is able to affect plasma AVP levels by 5 min further supports the idea that a peripheral action is involved, at least in part, in mediating the effect of morphine in plasma AVP.

In contrast plasma α-MSH levels were not significantly different from control values 5 min after ip administration of morphine yet icv administration was fully effective at this time. This points to a central action of morphine rather than a peripheral one in its effect in α-MSH release. Naltrexone is able to block the effects of morphine on plasma α-MSH and AVP levels after water deprivation and hypertonic saline, indicating that under these conditions the effects of morphine may be mediated by opiate receptors. The same holds for the elevation of α-MSH levels after β-endorphin administration. However, the observation that naltrexone did not block the inhibitory action of β-endorphin on plasma AVP levels, points to the involvement of other receptor systems than the opiates in this respect. This fits rather well with the ineffectiveness of naltrexone to change basal plasma levels of AVP (unpublished data). More extensive studies are needed to elucidate the precise physiological role of β-endorphin in the mechanism of AVP and α-MSH release.

There has been some debate as to whether PLG will inhibit MSH release. While some workers (22,29) have reported that this tripeptide is an MSH release inhibiting factor others have failed to confirm this (see 30). The present findings are interesting in that PLG had no effect on basal plasma

α-MSH levels but seems to reduce the β-endorphin induced rise in plasma α-MSH. Whether this was due to a central action or whether PLG was acting at the pituitary level remains to be seen.

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