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EFFECT OF CHRONIC MORPHINE TREATMENT ON THE ADRENALINE BIOSYNTHESIS IN ADRENALS AND BRAIN REGIONS OF THE RAT

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Abstract—Phenylethanolamine-*N*-methyltransferase (PNMT) activity and tissue catecholamine content were examined after 13 days morphine treatment. Prolonged morphine treatment did not alter the PNMT activity in brain regions (A1/C1 and A2/C2 cell groups, medial basal hypothalamus, median eminence). However, the enzyme activity and the adrenaline content were increased in the adrenal medulla of male rats. In parallel, morphine treatment induced adrenal hypertrophy. In female or hypophysectomized male animals the chronic morphine treatment had no effect on adrenal weight but evoked the increase of PNMT activity. It is concluded that the morphine-induced increased adrenaline biosynthesis in the adrenal gland is not fully dependent on the intact pituitary–adrenal axis and may be mediated partly by a neural mechanism (increased splanchnic nerve activity) or by a direct effect of morphine.

It has been reported that morphine evokes adrenaline release from adrenal medulla [1]. Chronic morphine treatment causes increased activity of tyrosine hydroxylase (TH)† and dopamine β-hydroxylase (DBH) in the adrenals and the neural input plays an important role in this effect [2]. It is well known that the control of these enzymes is predominantly trans-synaptic. However, there is a second control system which is mediated through the hypothalamo-pituitary–adrenal axis. This latter is a specially important way of regulation of the PNMT activity. In this respect it is interesting to find that morphine injection stimulates the secretion of ACTH as reflected by increased plasma levels of corticosterone in the rat [3, 4]. In addition, recently Heybach and Vernikos [5] reported that morphine can potentiate the adrenal steroidogenic response to ACTH. Formerly it was shown that long-term ACTH treatment induces adrenal hypertrophy and increase of the PNMT activity and the adrenaline content in the adrenals [6]. Tanabe and Cafruny [7] found that chronic morphine treatment causes adrenal hypertrophy in rats. These observations raised the question whether the morphine administration can influence the PNMT adrenal activity and if the changes induced by morphine are similar to those induced by ACTH. Since the glucocorticoids can influence the PNMT activity in the brain [8, 9] the effects of ACTH and morphine on the PNMT activity of different brain regions have been investigated as well.

MATERIALS AND METHODS

Male or female CFY Sprague–Dawley rats of 200–250 g body weight were used. Morphine–HCl was administered subcutaneously in increasing doses for 13 days. The starting dose was 2 × 5 mg/kg at 9 a.m. and 6 p.m., this dose was increased by 5 mg/kg on each day. The controls received saline. In hypophysectomized male rats the first morphine injection was given 10 days after surgery. In a separate experiment normal male rats were treated i.m. daily with ACTH (30 μg/rat, Humactid®, G. Richter Ltd., Budapest, Hungary) or saline (50 μl/rat) for 14 days.

The animals were killed by decapitation between 9 and 10 a.m. on the day after the last injection. After decapitation the adrenals and the brains were immediately removed, dissected and stored on dry ice. Medial basal hypothalamus and median eminence were prepared under microscopic control, the medullary regions were dissected by the method described previously [10].

PNMT activity was assayed by a modification on the method of Moore and Phillipson [8]. Adrenal glands and adrenal medullae were homogenized in approx. 100 vol. of 0.005 M Tris–HCl buffer (pH = 8.6) containing 0.2% Triton-X 100. Ten microlitres of the supernatant was pipetted in a tube containing the following reagents: 40 μl 0.25 M Tris–HCl (pH = 8.6), 10 μl 3.5 mM DL-phenylethanolamine or 0.01 N HCl (blank) and 10 μl of a solution containing 1 μCi/^{3}H/SAM (15 Ci/mmol, 1 mCi/ml), 1.26 nmol SAM/HSO_4/, 6.25 nmol of pargyline and 7 nmol of dithiothreitol in 0.25 M Tris–HCl (pH = 8.6). The tubes were incubated for 15 min at 37°, and the reaction was stopped by the addition of 130 μl 0.5 M borate–buffer (pH = 10). The radioactive product was extracted by 1 ml toluene–isoamyl-alcohol (97:3,

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† Abbreviations used: PNMT, phenylethanolamine-*N*-methyltransferase; TH, tyrosine hydroxylase; DBH, dopamine β-hydroxylase; ACTH, adrenocorticotropin.

v/v). After centrifugation 800 μl organic phase was transferred to tubes containing 80 μl of 0.1 N HCl. After shaking and centrifugation the organic phase was discarded and 50 μl of the aqueous phase was transferred to a scintillation vial and evaporated to dryness in a chromatographic oven at 60° to remove volatile radioactive contaminants. The residue was subsequently dissolved in 0.5 ml ethanol, then 5 ml scintillation fluid was added and the radioactivity was counted. The brain supernatants were incubated as described above with the following minimal modifications: 5.6 nM DL-phenylethanolamine were used, the mixture did not contain SAM/HSO_4 and the incubation time was 60 min. Under these conditions the reaction was linear with the time and with the protein concentration.

The catecholamine content was measured radioenzymatically [11, 12].

The protein content of supernatants was determined photometrically [13].

Statistical analysis was done using Student's *t*-test.

RESULTS

The prolonged morphine treatment of male rats induced adrenal hypertrophy and an increase of PNMT activity which is measurable in the whole gland and also in the medulla (Table 1).

There was an approximately 40% elevation of adrenaline content of adrenals of morphine-treated rats (Table 2). Dopamine and noradrenaline levels were also increased.

Hypophysectomy of male rats caused atrophy of adrenal gland, the adrenal weight decreased to 30% of the control, and the PNMT activity was also markedly reduced. While the chronic morphine administration resulted in an increase in adrenal weight of normal animals, it had no effect on adrenal weight of hypophysectomized rats. However, the PNMT activity of the whole adrenal gland was elevated by morphine in hypophysectomized rats, too (Fig. 1).

If female rats were treated with morphine the adrenal PNMT activity rose to 130% of the control value, but the adrenal weight did not alter (Table 3). The adrenal weight and the PNMT activity in control females are higher than in males.

The prolonged morphine administration was without effect on the PMT activity in the brain of male rats either in adrenergic cell body regions (A1/C1 and A2/C2 areas) or in areas rich in nerve terminals (medial basal hypothalamus and median eminence). On the other hand, the long-term ACTH treatment induced a significant increase of PNMT activity in each brain region investigated (Table 4).

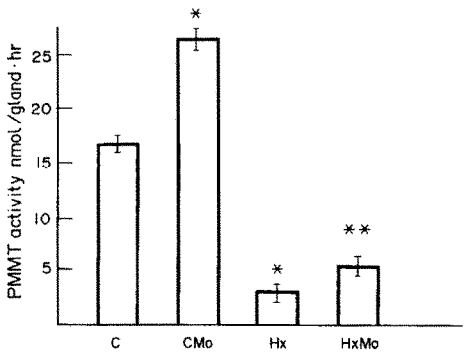

Fig. 1. PNMT activity in adrenals of control and hypophysectomized animals after 13 day's morphine or saline treatment. Points represent means ± SEM of 7–11 animals. C—control; HX—hypophysectomized; Mo—morphine-treated, * $P < 0.001$ vs C, † $P < 0.02$ vs HX.

DISCUSSION

The effect of prolonged morphine treatment on the adrenal PNMT activity can be mediated in several ways. (1) The chronic administration of morphine produces repeated increase in the endogenous level of the plasma corticosterone (in the medullary capillaries the corticosterone level is much higher than in the periphery), which leads to an increase of the adrenal PNMT activity. Morphine evokes secretion of catecholamines from the adrenal gland, this stimulation results in a compensatory increase of PNMT activity which may take place (2) by a neural way via nervus splanchnicus or (3) by a direct action of the opioid.

Table 2. Effect of prolonged morphine treatment on the catecholamine content in the adrenals of male rats (mean ± SEM, μg/gland values, N = 6)

	Control	Morphine-treated
Dopamine	0.98 ± 0.20	1.65 ± 0.20*
Noradrenaline	1.64 ± 0.20	2.63 ± 0.23†
Adrenaline	4.97 ± 0.46	6.65 ± 0.57*

* $P < 0.05$, † $P < 0.01$ compared to the respective controls.

Table 1. Effect of prolonged morphine treatment on the PNMT activity in the adrenals of male rats (mean ± SEM, N = 6)

Weight (mg)	Control 35.5 ± 1.7	Morphine-treated 50.1 ± 2.7*
PNMT activity, nmol × gland^{-1} × hr^{-1}	18.0 ± 0.4	26.5 ± 1.6†
PNMT activity, nmol × mg protein^{-1} × hr^{-1}	43.7 ± 2.8	55.7 ± 2.1*

* $P < 0.01$, † $P < 0.001$ compared to the respective controls.

Table 3. PNMT activity in adrenals of control and morphine-treated female rats

Weight (mg)	Control 53.7 ± 3.8 (7)	Morphine-treated 51.6 ± 3.9 (8)
PNMT activity, nmol × gland^{-1} × hr^{-1}	24.7 ± 2.2 (7)	31.7 ± 0.9 (8)*

Mean ± SEM (N).
* $P < 0.01$.

(1) The described morphine treatment of the animals caused a 43% increase in adrenal weight of normal male rats and evoked the elevation PNMT activity and adrenaline content in the adrenal. The PNMT activity of the adrenal medulla is regulated by glucocorticoids. Hypophysectomy leads to a decrease of the PNMT activity in the adrenal gland and this enzyme activity is restored to normal by injections of ACTH or dexamethasone [14, 15]. It has been generally accepted that in the intact animals the PNMT activity appears to be maximal so that hormone treatment does not elevate the enzyme activity above control level [15–18]. The inability of these investigators to show an increase of the PNMT activity in the intact rats is possibly due to the fact that the system may require prolonged stimulation rather than acute exposure to high levels of pituitary-adrenal activity. Compensatory hypertrophy [19], repeated immobilization [20] or long-term ACTH treatment [6] are equally effective to increase the enzyme activity in intact animals. In these experiments the increase of PNMT activity is always associated with adrenal hypertrophy. The adrenal denervation cannot prevent the elevation of enzyme activity induced by repeated immobilization showing an unambiguous effect of glucocorticoids. Therefore we may conclude that repeated increase of endogenous glucocorticoid level certainly plays a role in the effect of morphine. In the early phase of the treatment corticosterone may rise as a direct response to morphine; after development of tolerance, the increase is possibly due to repeated withdrawal.

The results of experiments in hypophysectomized or female rats are apparently inconsistent with the above conclusion: the morphine treatment did not induce adrenal hypertrophy, while the PNMT activity was elevated in these rats. However, female rats metabolize morphine more slowly than male rats [21] and might not experience the same degree of withdrawal as male rats. In addition, there are important sex differences in adrenal corticosteroid secretion in the rats [22]. This evidence can explain the lack of hypertrophy in females and confirm the role of steroids. In hypophysectomized animals the increase of PNMT activity cannot be mediated by glucocorticoids because morphine has no direct effect on adrenal steroidogenesis [5]. This suggests that morphine-caused increase of PNMT activity of adrenal is at least not totally dependent on the intact pituitary–adrenal axis.

(2) Concerning the possible neural or direct effect of morphine, Anderson and Slotkin [23] reported that chronic morphine treatment increased TH and DBH activities and catecholamine content of adrenals, most of these effects resulting from increased stimulation of the splanchnic nerve activity. The evidence that PNMT activity is influenced by the alteration in neural activity [18, 24, 25] suggests the possibility that the effect of morphine may partly result from trans-synaptic induction. However, the trans-synaptic regulation has been thought to be of less importance in the regulation of PNMT than in that of DBH or TH [20, 26].

(3) Yoshizaki [27] has demonstrated that morphine may exert a direct catecholamine-releasing effect in chronically denervated adrenal medulla. The fact that in the above-mentioned experiments of Anderson and Slotkin [2, 23] the effects of morphine are reduced but not abolished by adrenal denervation, leads to the conclusion that morphine exerts a direct effect on the catecholamine biosynthesis of adrenal. Therefore it is possible that the marked increase in PNMT activity of hypophysectomized animals is primarily due to a direct effect of morphine. However, in intact animals all three above-described mechanisms may take part in mediation of morphine's action; their relative importance remains to be elucidated.

In contrast to the marked effect of morphine on the adrenaline biosynthesis in the adrenals, chronic

Table 4. Effect of prolonged morphine or ACTH treatment on the PNMT activity in brain regions

Brain regions	PNMT activity Control	Morphine-treated	ACTH-treated
A1/C1 areas	27.06 ± 0.08 (6)	27.99 ± 0.18 (6)	40.02 ± 4.3 (8)*
A2/C2 areas	74.77 ± 6.84 (7)	86.35 ± 7.92 (5)	115.82 ± 11.84 (7)†
Medial basal hypothalamus	13.82 ± 1.37 (8)	12.92 ± 1.62 (4)	18.46 ± 1.62 (8)*
Median eminence	19.57 ± 2.82 (9)	25.17 ± 6.37 (5)	35.40 ± 3.59 (8)‡

Mean ± SEM, (N), pmol × mg protein^{-1} × hr^{-1} values.
* $P < 0.05$, † $P < 0.02$, ‡ $P < 0.01$.

morphine administration had no effect on the PNMT activity in brain regions. The explanation for this difference remains to be elucidated. A similar difference was found regarding TH activity [28]; therefore it seems to be a difference between the mechanisms regulating enzyme activities of catecholamine biosynthesis in the brain and in the adrenals. A similar conclusion was drawn by Turner *et al.* [29, 30] investigating stress-induced increase of PNMT activity.

The long-term ACTH treatment induced a dramatic increase of PNMT activity in brain regions. This effect of ACTH is presumably mediated by glucocorticoids, because a similar increase can be induced by chronic dexamethasone administration [24]. These results are not in agreement with those of Parvez *et al.* [9]. Our formerly published data showed that ACTH treatment increased the adrenaline synthesis in the adrenals, too [6]. These findings, together with the results of others [30–32], emphasize the importance of prolonged stimulation of the pituitary–adrenal axis in the induction of adrenaline biosynthesis.

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