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THE ROLE OF NEURAL INPUT IN THE EFFECTS OF MORPHINE ON THE RAT ADRENAL MEDULLA*

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Abstract—Rats with denervated left and intact right adrenal glands were treated with morphine twice daily and the individual adrenals were analyzed for catecholamine (CA) content and for tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) activities. After one day of treatment at a low dosage level (10 mg/kg) neither side demonstrated any change in CA, but DBH was increased in both; TH was elevated only in the intact side. After one week of treatment, CA levels were elevated only slightly in both intact and denervated adrenals, while the effect on DBH was more marked (30 per cent elevation). While TH was increased in the innervated side by more than 50 per cent, there was only a small increase in TH in the denervated adrenal. With the subsequent initiation of higher dosages, there was an initial decline in CA which was more pronounced in the innervated gland, accompanied in the denervated side by short-term decreases in DBH. Chronic administration of 40 mg/kg or 100 mg/kg led to larger increases in CA, TH and DBH in the innervated glands. In the denervated adrenal there were only slight increases in CA and TH after chronic administration of the higher doses, while DBH activity was markedly elevated during chronic administration of 40 mg/kg but declined toward control levels during chronic treatment with 100 mg/kg.

These data indicate that: (1) most of the morphine-induced increases in TH activity and CA content result from increased stimulation of the splanchnic nerve; (2) morphine also exerts a direct effect on the adrenal medulla which influences DBH activity primarily; (3) the direct action of morphine may display tolerance while the increased splanchnic stimulation does not.

The release, synthesis and storage of catecholamines in the sympatho-adrenal axis are subject to a number of regulatory mechanisms which are designed to maintain transmitter levels in the face of situations in which there is increased sympathetic tone. Thus, administration of agents such as morphine [1], reserpine [2], 6-hydroxydopamine [3] or insulin [4], cold or immobilization stress [5, 6], or sinoaortic denervation [7] all evoke catecholamine-release from sympathetic tissues like the adrenal medulla but also cause increased levels of the catecholamine biosynthetic enzymes, tyrosine hydroxylase and dopamine β-hydroxylase and accelerated synthesis of storage vesicles. It is thought generally that the predominant mode of control is trans-synaptic, i.e. that in the adrenal medulla the key signal is neural input via the splanchnic nerve; this simple model for drug action in the sympatho-adrenal axis has been complicated by the discovery of a second control system which is mediated through the hypothalamo-anterior pituitary-adrenocortical axis [8, 9]. In addition, Yoshizaki [10] has suggested that agents like morphine may exert a direct catecholamine-releasing effect in an adrenal medulla which has been denervated several days before drug exposure; neonatal rats, which do not have a functional nerve supply to the adrenal medulla [11] still display a reduction in catecholamines upon morphine treatment [12], further suggesting actions of the drug exclusive of trans-synaptic effects.

The current studies with denervated adrenals were undertaken to determine (1) whether morphine does indeed exert non-neural effects on the sympatho-adrenal axis, and (2) whether regulatory mechanisms for catecholamine release, synthesis and storage can function in the absence of trans-synaptic signals.

MATERIALS AND METHODS

Male Sprague–Dawley rats (Zivic Miller) with the left adrenal glands denervated 10 days previously were given morphine-HCl subcutaneously twice daily for 4 weeks. The dose was 10 mg/kg for the first week, 40 mg/kg for the second week, and 100 mg/kg for the third and fourth weeks, after which time the injections were stopped to initiate withdrawal. Control rats received the same surgical procedure, but were injected with saline. Animals were killed by decapitation at 24 hr or 6 days after each dosage increment, at 2 weeks after the initiation of 100 mg/kg, and at 24 hr and 3 days after discontinuing morphine. Individual adrenal glands were excised, cleaned of fat and homogenized glass-to-glass in 2.5 ml of 300 mM sucrose containing 25 mM Tris (pH 7.4) and 0.01 mM iproniazid. One-tenth-ml aliquots of the homogenate were removed, deproteinized with perchloric acid (final concentration, 3.5 per cent) and centrifuged for 10 min at 26,000 *g*. The supernatants were analyzed for catecholamines by the trihydroxyindole method using an autoanalyzer [13]. Duplicate 0.2-ml portions of the homogenate were added to 0.2 ml of water containing 2000 U of catalase and assayed for dopamine β-hydroxylase (periodate oxidation method [14], using [^{3}H]tyramine (10 μM) as substrate. *p*-Hydroxymercuribenzoate (0.5 mM) was used to inactivate endogenous inhibitors [15]. The remainder of the homogenates was centrifuged at 26,000 *g* for 10 min, and duplicate 0.1-ml portions of the supernatants

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were assayed for tyrosine hydroxylase activity by the method of Waymire *et al.* [16], using [^{14}C]tyrosine (100 μM) as substrate.

Results are presented as means ± S.E.M., and levels of significance calculated by Student's *t*-test [17].

Materials. Tyramine-[G-^{3}H] and tyrosine-[1-^{14}C] were purchased from New England Nuclear Corporation. Epinephrine bitartrate was obtained from Winthrop Laboratories and morphine hydrochloride from Merck, Sharp & Dohme.

RESULTS

Low doses of morphine (10 mg/kg) had no initial (24 hr) effect on catecholamine levels (Fig. 1); however, 24 hr after each subsequent increase in dose there was a 10–15 per cent depletion of catecholamines in both denervated and intact glands when the results were compared to the level at the previous point. Maintenance of each dose level for a period of several days resulted in an increase in catecholamine content of intact glands with a much smaller effect in denervated glands. The maximum effect achieved in intact adrenals was about 50 per cent elevation after chronic administration of either 40 or 100 mg/kg, while increases in the denervated side never exceeded 15 per cent and often were not statistically significant. After discontinuing morphine, catecholamine content in the denervated gland returned to the control level within 3 days; in the intact gland, the level of catecholamines was still slightly elevated (13 per cent) compared to controls after 3 days.

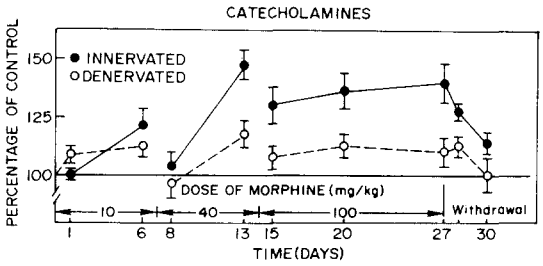

Fig. 1. Effects of morphine administration and withdrawal on catecholamines in intact and denervated rat adrenals. Points and bars represent means ± S.E.M. of 6 animals. Control values (66 animals) were: intact gland, 13.1 ± 0.4 μg/gland; denervated gland, 8.36 ± 0.22 μg/gland.

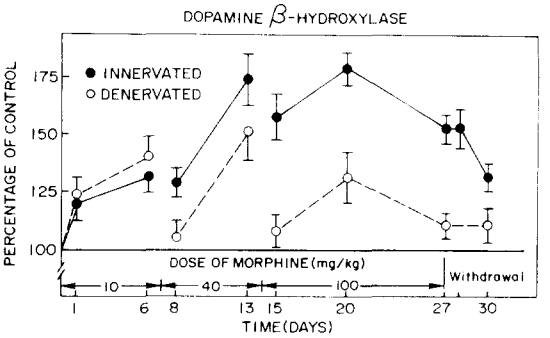

Fig. 2. Effects of morphine administration and withdrawal on dopamine β-hydroxylase activity in intact and denervated rat adrenals. Points and bars represent means ± S.E.M. of 6 animals. Control values (66 animals) were: intact gland, 1.29 ± 0.05 nmoles/gland/hr; denervated gland, 0.89 ± 0.03 nmoles/gland/hr.

Adrenal DBH activities in both innervated and denervated sides were increased by about 20 per cent 24 hr after initiation of administration of 10 mg/kg of morphine and by 30–40 per cent after 6 days (Fig. 2). When the dose was increased to 40 mg/kg, there was an initial decline in DBH in the denervated side, but after several days at this dose level DBH in both sides were elevated by 50–75 per cent. Within 24 hr of initiation of 100 mg/kg, DBH in the denervated gland fell nearly to control levels while there was little or no decline in the innervated adrenal; after 6–14 days at 100 mg/kg DBH activity in the intact side remained elevated by 50–80 per cent while in the denervated gland there was only a 30 per cent elevation at 6 days and no significant elevation after 14 days. Within 3 days of discontinuing morphine administration, DBH levels in the innervated gland began to decline toward control, but were still elevated compared to the denervated adrenal.

Because newly-formed vesicles contain below-nor-

Table 1. Effects of morphine administration on the ratio of catecholamines to dopamine β-hydroxylase in intact and denervated rat adrenals

Day no.	Dose of morphine (mg/kg)	Catecholamines/dopamine β-hydroxylase (percentage of control)	
		Innervated	Denervated
0	0	100 ± 3	100 ± 3
1	10	85 ± 6*	86 ± 2†
6	10	87 ± 8	79 ± 2†
8	40	81 ± 2†	90 ± 3*
13	40	86 ± 4‡	66 ± 12‡
15	100	82 ± 8*	99 ± 9
20	100	76 ± 2†	93 ± 5
27	100	95 ± 3	100 ± 2
28	0	80 ± 3†	74 ± 2†
30	0	86 ± 1†	91 ± 8

Data represent means ± S.E.M. of 6 animals at each point. Control values (66 animals) were: innervated side, 10.5 ± 0.3 μg CA per unit of DBH; denervated side, 9.88 ± 0.32 μg per unit. Dosage schedule is described in Materials and Methods.

* $P < 0.05$ vs. control

† $P < 0.001$ vs. control

‡ $P < 0.01$ vs. control

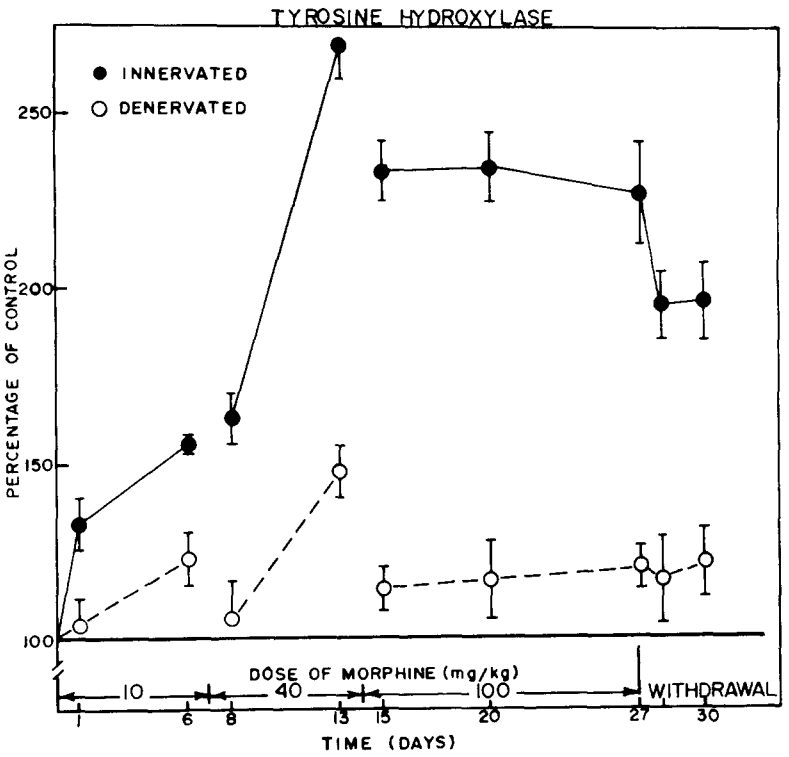

Fig. 3. Effects of morphine administration and withdrawal on tyrosine hydroxylase activity in intact and denervated rat adrenals. Points and bars represent means ± S.E.M. of 6 animals. Control values (66 animals) were: intact gland, 10.9 ± 0.4 nmoles/gland/hr; denervated gland, 7.78 ± 0.28 nmoles/gland/hr.

mal catecholamine contents but normal levels of proteins, the ratio of catecholamines to DBH is in part a measure of the relative rates of amine synthesis and formation of new vesicles [1, 11, 12]. In innervated glands, the ratio was low throughout the course of morphine administration except after 2 weeks at 100 mg/kg (day 27; Table 1); in contrast, the denervated glands demonstrated normal ratios by the first day of treatment with 100 mg/kg (day 15). In both sides, the initiation of withdrawal again produced a decline in the CA:DBH ratio.

The effects of morphine on tyrosine hydroxylase (TH) activity are shown in Fig. 3. In the intact adrenals, TH activity was markedly increased at all times and by all doses of morphine, reaching a maximum of about three times control at 40–100 mg/kg. The denervated adrenals showed much smaller elevations (maximum of 40 per cent, typically about 20 per cent), which often were not statistically significantly above control levels. Cessation of morphine administration produced after 3 days a partial decline toward control values in the innervated side.

DISCUSSION

The actions of morphine on the intact adrenal medulla comprise at least three types of effect [1]: first, morphine evokes secretion of catecholamines; second, stimulation results in compensatory induction of tyrosine hydroxylase and dopamine β-hydroxylase; and third, there is an acceleration of storage vesicle synthesis and formation of 'immature' vesicles with abnormally low ratios of catecholamines to dopamine β-hydroxylase. Classically, the effects on secretion and on enzymes have been attributed to reflex stimulation of the splanchnic nerve and consequent transsynaptic induction [18, 19]; however, Yoshizaki [10] has demonstrated a direct catecholamine-releasing effect of morphine in chronically denervated adrenals. The current study confirms the partial non-neural nature of morphine-induced secretion, since in both innervated and denervated adrenals there was a decrease in adrenal catecholamines 24 hr after initiation of administration of 40 mg/kg or 100 mg/kg. The observation of a direct component of secretion in chronically denervated adrenals may also explain why morphine causes a depletion of catecholamines in newborn rats, where splanchnic innervation is not yet functional [12].

The recovery of catecholamine stores after depletion is dependent in part upon the activity of the rate-limiting enzyme, tyrosine hydroxylase [20]. In intact adrenals, catecholamine levels after chronic morphine administration were markedly elevated, reflecting the 3-fold increase in tyrosine hydroxylase activity; on the other hand, catecholamine levels in denervated adrenals increased only slightly, reflecting the much smaller degree of tyrosine hydroxylase induction. These data confirm the previous observation [19] that acute morphine administration induces tyrosine hydroxylase primarily by a trans-synaptic mechanism, and also that the trans-synaptic increases do not display tolerance [1]. The small degree of tyrosine hydroxylase induction in denervated glands may be a direct effect of morphine, but mediation via ACTH from morphine-induced stimulation of the hypothalamo-pituitary axis [8, 21–23] cannot be ruled out.

In contrast to the marked neural dependence of

morphine-induced increases in adrenal catecholamines and tyrosine hydroxylase activity, dopamine β-hydroxylase activity in denervated glands kept pace with the increases in the innervated side after chronic administration of 10 or 40 mg/kg. It is unlikely that ACTH secretion alone is responsible for all of the increase: while hypophysectomy decreases adrenal dopamine β-hydroxylase levels which are partially restored by ACTH administration, in animals with intact pituitaries ACTH given for 5 days produces little or no elevation of dopamine β-hydroxylase activity [21], nor does ACTH increase tyrosine hydroxylase activity in non-hypophysectomized rats [23]. In the present study, morphine treatment resulted in an elevation in DBH activity as early as 24 hr after the first injection, an effect which cannot be duplicated by ACTH administration. Thus, in addition to catecholamine secretion, the increase in dopamine β-hydroxylase activity may represent a direct effect of morphine on the adrenal medulla. It is of special interest that while the dopamine β-hydroxylase induction after chronic morphine administration in innervated glands does not display tolerance, the directly evoked increase in denervated glands disappears after chronic administration of high doses (contrast days 6, 13, 20 and 27 in Fig. 2). The ability to increase dopamine β-hydroxylase activity in the absence of neural input is not peculiar to morphine, since chronic administration of at least two other agents, reserpine [4] and chlorisondamine [24] can evoke increases in denervated glands.

Since dopamine β-hydroxylase is a marker enzyme for storage vesicles, these data suggested that the morphine-induced increase in storage vesicle synthesis could occur in the absence of neural input. One index of increased vesicle formation is the catecholamine:dopamine β-hydroxylase ratio [20, 25, 26], which depends upon the relative rates of synthesis of amines and storage vesicles. Typically, new vesicle synthesis precedes increases in amine levels, resulting in a fall in the ratio when the tissue is stimulated [1, 11, 20, 25]. Additionally, exocytotic secretion itself reduces the catecholamine to dopamine β-hydroxylase ratio because empty vesicle membranes with enzyme activity remain behind after secretion [20, 25, 26]. However, even after massive secretion of adrenal vesicle content evoked by insulin, most of the remaining empty vesicle membranes are destroyed within 24 hr [20]. Thus, in long-term studies with morphine, which causes a much smaller degree of secretion than insulin, it is not likely that the increased dopamine β-hydroxylase activity and reduced catecholamine:dopamine β-hydroxylase ratios result from accumulated empty membranes; indeed, it has been shown earlier [1] that the chronic morphine-induced drop in catecholamines/dopamine β-hydroxylase corresponds to an increase in the number of new, intact storage vesicles.

In the current study, both innervated and denervated glands demonstrated a fall in the catecholamine:dopamine β-hydroxylase ratio over the first 2 weeks of treatment, indicating that the increased enzyme activity probably represents accelerated formation of vesicles. The denervated glands, however, displayed a normal ratio over the following 2-week period (the period during which tolerance to the increase in dopamine β-hydroxylase developed), while the innervated glands maintained a low ratio at least 1–2 weeks longer. The recovery of the ratio in the innervated glands occurred at a time when both catecholamines and dopamine β-hydroxylase were still elevated, indicating that in these glands, recovery resulted not from tolerance, but rather from the fact that amine synthesis 'caught up' to vesicle synthesis. These studies suggest that the rate of synthesis of storage vesicles is subject to direct as well as trans-synaptic control, and that while the former mechanism displays tolerance to morphine, the latter does not.

In conclusion, it is evident that morphine exerts both direct and neurally-mediated effects on catecholamine synthesis, storage and release in the adrenal medulla. Direct effects consist primarily of morphine-evoked catecholamine secretion and increased storage vesicle synthesis (dopamine β-hydroxylase activity) and may display tolerance; neurally-mediated effects include the above factors plus tyrosine hydroxylase induction and increased catecholamine levels and do not display tolerance.

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