Biochemical Pharmacology, Vol. 24, pp. 671–679. Pergamon Press. 1975. Printed in Great Britain.

EFFECTS OF MORPHINE ON THE RAT ADRENAL MEDULLA*

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(*Received* 27 *May* 1974; *accepted* 26 *August* 1974)

Abstract—Morphine was administered twice daily to rats and the adrenals were analyzed for catecholamines (CA), tyrosine hydroxylase (TH) activity and dopamine β-hydroxylase (DBH) activity. Twenty-four hr after dosage with 10 mg/kg, CA were reduced and TH and DBH were slightly elevated. After 1 week of treatment, all three were elevated. Subsequent dose increments resulted in acute decreases in CA and DBH and increases in TH, followed by dose-dependent increases in CA, DBH and TH to a maximum of two to three times control levels after 2 weeks at 100 mg/kg. Withdrawal led to a decline to control levels within 10 days. Shifts in the subcellular distribution of DBH suggested that morphine administration increased the rate of vesicle synthesis, and measurements of the uptakes of [^{14}C]epinephrine and [^{3}H]metaraminol into isolated storage vesicles indicated an increased proportion of "immature" vesicles. Prolonged administration of large doses of morphine led to the formation of vesicles with an apparently defective amine uptake mechanism; these vesicles also displayed abnormally low fragility and a reduced rate of spontaneous CA efflux. These data suggest that: (1) tolerance to morphine-induced sympathoadrenal discharge does not develop and that the recovery from the acute CA depletion results from increased CA synthesis and storage; and (2) morphine produces a persistent change in the properties of adrenal medullary storage vesicles.

The stimulatory effect of morphine on the adrenal medulla was first noted by Elliott 60 years ago [1]. Since then, a number of investigators have shown that acute morphine administration results in a decrease in adrenal catecholamines but that, with chronic treatment, amine levels return to normal and in some instances to supranormal levels [2–12]. These results have led to the conclusion that tolerance develops to the sympatho-adrenal stimulation in a fashion similar to the analgesic and hypothermic actions of morphine. However, in these studies it was not determined whether the recovery of catecholamine stores represented a cessation of stimulation or increased synthesis of catecholamines. Similar results have been reported for the effects of morphine on catecholamine disposition in the central nervous system [8, 13]. However, Clouet and Ratner [14] have shown that the incorporation of [^{14}C]tyrosine into dopamine and norepinephrine in rat brain is increased by morphine, and that tolerance to the accelerated synthesis does not develop; these results have been challenged by Smith *et al.* [15]. While the adrenal probably does not play a role in morphine dependence or analgesia [16], the biochemical similarities between the adrenal medulla and adrenergic neurons suggest that this might serve as a model system with which to study the effects of morphine on catecholamine disposition.

In the current experiments, the effects of acute and chronic morphine administration on the adrenal medulla have been determined by measurements of biochemical parameters which evaluate the synthesis, uptake, storage and release of catecholamines.

METHODS

Morphine HCl was administered subcutaneously twice daily to male Wistar rats (Hilltop Lab Animals) weighing 200–300 g. The dose was 10 mg/kg for the 1st week, 40 mg/kg for the 2nd week and 100 mg/kg for the 3rd and 4th weeks, at which time injections were discontinued to initiate withdrawal. In initial experiments, another set of rats received injections of water on the same schedule, and were compared to untreated animals; in none of the measured parameters were differences found between untreated and water-injected animals, and in subsequent experiments controls received no injections. Animals were killed by decapitation at 24 hr or 1 week after each dosage increment, at 2 weeks after initiation of 100 mg/kg, and at 24 hr, 3, 5, 10 and 17 days after withdrawal. The adrenal glands from each animal were excised, cleaned of fat and connective tissue, and homogenized (glass-to-glass) in 2·5 ml of ice-cold 300 mM sucrose containing

* Supported by United States Public Health Service Grant DA-00465-01.

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25 mM Tris (pH 7·4) and 0·01 mM iproniazid (irreversible monoamine oxidase inhibitor) (sucrose–Tris). The suspension was centrifuged at 800 *g* for 10 min, the pellet (fraction A) was resuspended in 5 ml of water, and an aliquot was assayed for dopamine β-hydroxylase (DBH) activity. Another aliquot was deproteinized with 3·5% perchloric acid (PCA), centrifuged and assayed for catecholamines (CA). The 800 *g* supernatant was divided into three aliquots: 0·6 ml was added to an equal volume of water and assayed for CA and DBH (fraction B); 0·5 ml was centrifuged for 10 min at 26,000 *g* and the pellet (fraction P) was homogenized in 1 ml of 3·5% PCA and assayed for CA, while the supernatant (fraction S) was assayed for CA and tyrosine hydroxylase (TH); 1 ml of the 800 *g* supernatant was layered over 2·5 ml of 1·6 M sucrose (buffered at pH 7 with 10 mM Tris) containing 500 units/ml of beef catalase (Sigma) and centrifuged at 140,000 *g* for 2 hr in the No. 40 rotor of the Beckman model L5-50 ultracentrifuge. This latter procedure separates intact heavy vesicles from broken vesicle membranes [17], from most contaminants [18] and, to some extent, from intact vesicles of lower density [17, 19, 20]. The 0·3 M sucrose layer (fraction C) and the 1·6 M sucrose layer (fraction D) were each diluted with water to a final volume of 2 ml and assayed for CA. Fraction C was also assayed for DBH. The vesicular pellet (fraction E) was homogenized in 2 ml water to lyse the vesicles. An aliquot of fraction E was centrifuged at 26,000 *g* to remove the vesicle membranes, which contain an ATPase, and assayed for ATP. Another aliquot of fraction E was used for the determination of CA and DBH.

Uptake of amines. Rats were given morphine and killed as described. The glands of each animal were homogenized in 3·0 ml sucrose–Tris–iproniazid and an aliquot was removed for CA assay. The suspension was centrifuged at 800 *g* for 10 min, and 0·5 ml of the supernatant was added to each of four tubes containing 5 mM ATP-Mg^{2+}, 0·1 mM epinephrine and either 1 μCi [^{14}C]epinephrine or 5 μCi of 0·1 mM [^{3}H]metaraminol. The unlabeled epinephrine was added to obviate any differences in extravesicular catecholamine concentrations among the samples. Sucrose–Tris was added to bring the volume of each tube to 1 ml. One epinephrine- and one metaraminol-containing sample were brought to 30° for 30 min; the duplicate tubes were kept on ice. Uptake was stopped by adding 2 ml of ice-cold sucrose–Tris. The samples were centrifuged at 26,000 *g* for 10 min, and aliquots of the supernatants were assayed for CA and radioactivity after deproteinization with PCA. The vesicular pellets were washed and recentrifuged twice with sucrose–Tris, and the final pellets were resuspended in 3 ml of 3·5% PCA and analyzed for CA and radioactivity. Under these conditions, labeling occurs solely in storage vesicles [20]. The temperature-dependent component of uptake in each sample was calculated as described previously [21].

To determine whether morphine exerted a direct effect on amine uptake *in vitro*, the uptakes of epinephrine and metaraminol were determined in vesicle suspensions from untreated rats as described above, except that morphine was added to the incubation tubes in final concentrations ranging from 0·1 to 100 μM and compared to uptake in the absence of morphine.

Efflux of amines. The glands of four control rats or four morphine-treated rats were homogenized in 14 ml of sucrose–Tris–iproniazid and an aliquot was removed for CA analysis. The homogenate was centrifuged at 800 *g* for 10 min and the supernatant was divided into 12 1-ml portions. Six of the aliquots were kept at 0°, and six were incubated at 30° to allow efflux to occur. The efflux was stopped after 10 min or 1 hr by addition of ice-cold sucrose–Tris; the samples were centrifuged at 26,000 *g* for 10 min, and the supernatant and pellet analyzed for CA. The efflux of CA was calculated as described previously [21].

To determine whether morphine could exert a direct effect on efflux, vesicles from untreated rats were labeled with [^{3}H]epinephrine and the effluxes of labeled and endogenous amines were measured in the presence of either 1 or 100 μM morphine and compared to efflux in the absence of morphine.

Effects of morphine on enzyme activities in vitro. Adrenals from six untreated rats were homogenized in 12 ml of 0·15 M KCl. An aliquot of the homogenate was centrifuged at 26,000 *g* for 10 min and the supernatant was used for the assay of TH. The remaining homogenate was used for the assay of DBH and monoamine oxidase (MAO). Hog kidney was used as a source of dopadecarboxylase (DDC) prepared by the method of Waymire *et al.* [22]. The effects of morphine in concentrations up to 100 μM were determined for each enzyme.

Effects of morphine on insulin-induced depletion of adrenal catecholamine stores. Control rats or rats which had been given morphine (100 mg/kg) for 2 weeks were fasted 24 hr, given insulin (5 i.u./kg) via a tail vein, and killed 3 hr later. The adrenal glands from each rat were homogenized in 3·5% PCA, centrifuged at 26,000 *g* and analyzed for CA.

Analytical procedures. DBH activity was assayed by the method of Friedman and Kaufman [23], using [^{3}H]tyramine (10 μM) as substrate. *para*-Hydroxymercuribenzoate (PMB) was used to inactivate endogenous inhibitors [24]; optimal PMB concentrations were: fraction A, 1 mM; fractions B and C, 0·5 mM; fraction E, 0. DBH activity generally was not determined in fraction D because of the low levels of activity found [17]. TH and DDC activity were measured by the method of Waymire *et al.* [22], using [^{14}C]tyrosine (100 μM) or [^{14}C]DOPA (33 μM) as substrates. MAO activity was measured by the method of Laduron and Belpaire [25], using [^{3}H]tyramine (10 μM) as substrate.

CA were analyzed by the trihydroxyindole method using an autoanalyzer [26], and radioactive amines were measured by liquid scintillation spectrometry as described previously [21]. ATP was analyzed by a

modification of the luciferin–luciferase method [27] as described previously [17]; phosphorescence was determined in a Farrand fluorometer with the filters removed 20 sec after the addition of the ATP-containing sample.

Statistical analyses. Data are reported in terms of control values and percentages of control. Levels of significance were calculated by Student's *t*-test [28].

Materials. Epinephrine-7-[^{14}C], epinephrine-7-[^{3}H], dopa-1-[^{14}C], metaraminol-7-[^{3}H], tyramine-G-[^{3}H] and tyrosine-1-[^{14}C] were purchased from New England Nuclear Corp. Buffered firefly extract was obtained from Worthington Biochemicals. Epinephrine bitartrate was obtained from Winthrop Laboratories, metaraminol bitartrate and morphine hydrochloride from Merck, Sharp & Dohme, and regular insulin (80 units/ml) from Squibb.

RESULTS

Effects of morphine on CA, TH and DBH (Fig. 1). Twenty-four hours after the administration of 10 mg/kg of morphine, there was a 10 per cent decrease in total CA ($P < 0.05$) and a small increase ($P < 0.05$) in TH and DBH activities. However, after 1 week at this dose, CA, DBH and TH were all significantly increased. When the dose was increased to 40 mg/kg, there was an initial decline in CA and DBH toward control levels, but a further increment in TH activity to more than twice the control value. After 1 week at 40 mg/kg, all three parameters were once again elevated. A similar pattern was obtained at 100 mg/kg: there was an initial decline in CA and DBH but an increase in TH, and all parameters were elevated after 1 week at this dose. Up until 2 weeks at 100 mg/kg, the increases in both enzymes always exceeded the increment in CA; however, after 2 weeks at the highest dose, both CA and DBH were equally elevated (180 per cent of controls) and TH was nearly tripled.

Twenty-four hours after the start of withdrawal (day 29), CA, DBH and TH had all started to decline. The decline in CA and DBH (but not TH) leveled off somewhat between 3 and 5 days after the commencement of withdrawal, and all three reached control levels by 10 days (day 38); however, the period required to return to control values appeared to vary somewhat among groups of rats, and in some experiments the return to control levels required more than 10 days.

Effects of morphine on subcellular distributions of CA and DBH. The subcellular distribution of CA is shown in Table 1. In control animals, approximately 12 per cent of the total CA was found in the 800 *g* pellet (A), 40 per cent in the two layers of the 140,000 *g* supernatant (C + D) and 50 per cent in the pellet (E). These values agree with those obtained previously [17, 18].

Twenty-four hours after the administration of morphine (10 mg/kg), the CA levels in all subcellular fractions were reduced (Table 1). After 1 week at 10 mg/kg, CA levels in all fractions were elevated. This pattern was repeated with each dosage increment—an initial fall in CA in each fraction followed by subsequent increases. After 2 weeks at 100 mg/kg, CA levels ranged from 165–230 per cent of control levels in the subcellular fractions.

Upon withdrawal, CA levels declined in all fractions, reaching control levels by 10 days of withdrawal (day 38); there was no further change at subsequent times (Table 1).

Throughout morphine administration and withdrawal, there was little or no change in the CA/ATP ratio in the purified heavy veiscle fraction (Table 1).

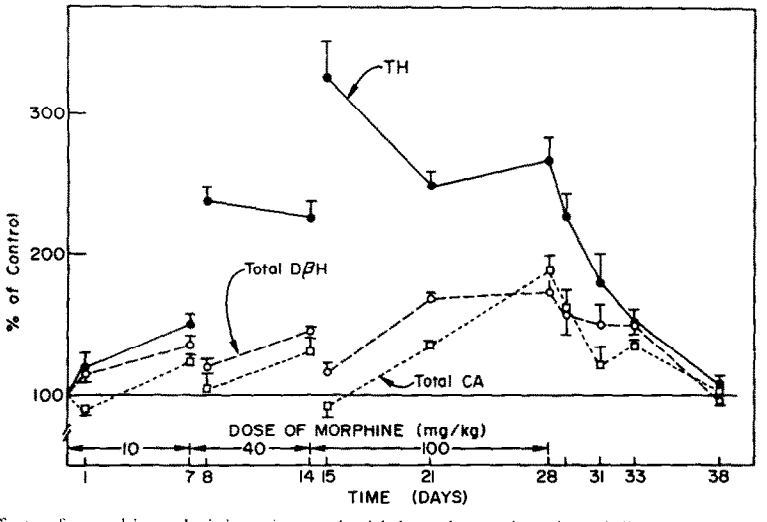

Fig. 1. Effects of morphine administration and withdrawal on adrenal medullary catecholamines (CA), tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH). Points and bars represent means ± standard errors of 5–12 animals. Control values (45 animals) were: catecholamines, 14·9 ± 0·4 μg/gland; tyrosine hydroxylase, 2·58 ± 0·2 nmoles/gland/hr; dopamine β-hydroxylase, 1·62 ± 0·07 nmoles/gland/hr.

Table 1. Subcellular distribution of catecholamines and ATP

Day no.	Dose of morphine (mg/kg)	A* (% control)	B (% control)	C + D (% control)	E (% control)	Molar ratio CA/ATP	No. of animals
	Control (μg/gland)	1·79 ± 0·06	12·4 ± 0·5	6·15 ± 0·16	6·98 ± 0·25		45
0	0	100 ± 3	100 ± 4	100 ± 3	100 ± 4	3·96 ± 0·05	45
1	10	76 ± 3†	92 ± 4	84 ± 4‡	94 ± 4	4·34 ± 0·19	6
7	10	129 ± 7†	125 ± 6§	119 ± 7‖	110 ± 6	3·76 ± 0·11	6
8	40	118 ± 11	102 ± 8	99 ± 2	107 ± 9	3·94 ± 0·09	5
14	40	151 ± 6†	127 ± 5†	148 ± 13†	132 ± 5†	3·99 ± 0·07	6
15	100	124 ± 8¶	89 ± 8	88 ± 3¶	96 ± 11	4·10 ± 0·26	5
21	100	170 ± 7†	128 ± 2†	130 ± 2†	140 ± 7†	3·70 ± 0·08¶	12
28	100	232 ± 12†	183 ± 9†	166 ± 4†	187 ± 15†	4·06 ± 0·05	6
29	0	229 ± 17†	156 ± 12†	151 ± 12†	160 ± 10†	4·03 ± 0·06	6
31	0	169 ± 13†	115 ± 12	121 ± 5†	104 ± 8	4·25 ± 0·12**	6
33	0	176 ± 6†	128 ± 3†	151 ± 10†	128 ± 5†	4·15 ± 0·06	6
38	0	102 ± 4	104 ± 3	92 ± 4	107 ± 4	3·76 ± 0·08	6
45	0	95 ± 4	102 ± 1	99 ± 2	100 ± 3	4·14 ± 0·27	6

* A, B = 800 *g* pellet and supernatant respectively; C, D, E = discontinuous sucrose density gradient (C = 0·3 M sucrose layer; D = 1·6 M sucrose layer; E = pellet). The ratio of CA/ATP was determined in fraction E. Values for A–E are given as means ± standard errors of the percentages of the control value.

† $P < 0·001$ vs control.

‡ $P < 0·005$.

§ $P < 0·002$.

‖ $P < 0·02$.

¶ $P < 0·01$.

** $P < 0·05$.

The subcellular distribution of CA after differential centrifugation in isotonic sucrose at 26,000 *g* was altered by morphine administration. Since this procedure sediments intact vesicles of all densities as well as vesicle membranes, the catecholamines in the supernatant (S) represent the fraction of CA released by lysis of the vesicles during preparation (mechanical fragility). The percentage of broken vesicles can thus be calculated as the ratio of CA in S × 100/(S + P). In control rats, CA from broken vesicles amounted to 16 ± 1 per cent of the total. As shown in Fig. 2, morphine administration tended to reduce the fragility of

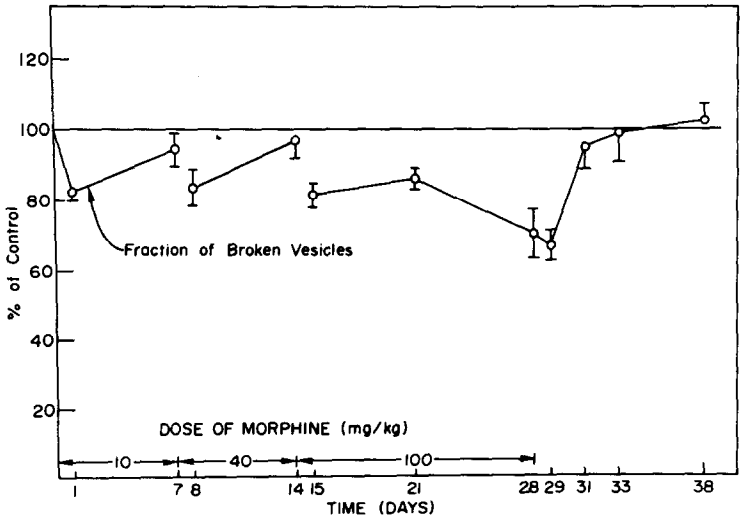

Fig. 2. Effects of morphine administration and withdrawal on mechanical fragility of storage vesicles as determined by differential centrifugation. Points and bars represent means ± standard errors of 5–12 animals. Control value (45 animals) for catecholamines found in the 26,000 *g* supernatant was 16 ± 1 per cent of total catecholamines in the 800 *g* supernatant fraction.

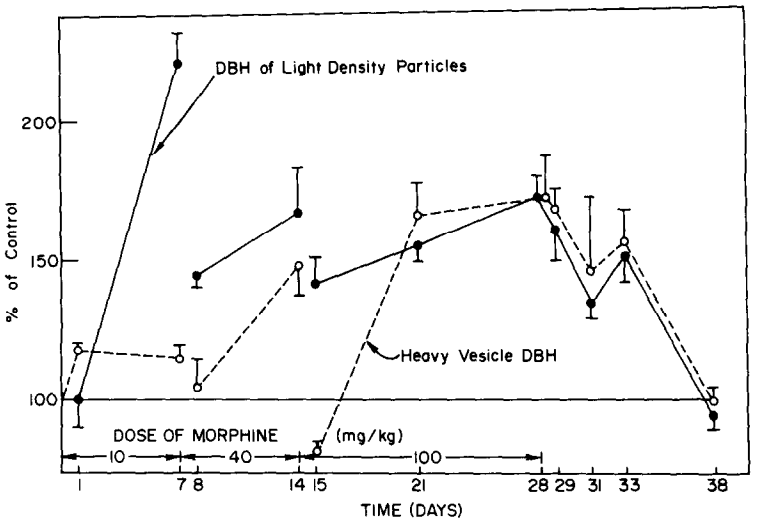

Fig. 3. Effects of morphine administration and withdrawal on the subcellular distribution of dopamine β-hydroxylase (DBH) in discontinuous sucrose density gradients. Light density particles are those which do not penetrate 1·6 M sucrose; heavy vesicles are those which sediment in 1·6 M sucrose. Points and bars represent means ± standard errors of 5–12 animals. Control values (54 animals) were: light density particles, 0·54 ± 0·02 nmoles/gland/hr; heavy density particles, 0·53 ± 0·02 nmoles/gland/hr.

the vesicles, and the effect was most pronounced after 2 weeks at the highest dose. Upon withdrawal, vesicle fragility returned to normal.

The subcellular distribution of DBH was also evaluated by the discontinuous gradient method. Recoveries averaged 80 per cent of the amount placed on the gradient. To some extent, both the morphine-induced changes in DBH associated with light density particles (fraction C, containing broken vesicle membranes as well as intact, light vesicles) and that associated with intact heavy vesicles (fraction E) mirrored the alterations in total DBH activity: a small or no change 24 hr after 10 mg/kg, an increase upon chronic administration, subsequent decrease toward control levels 24 hr after each increment in dose and further increases on continued administration (Fig. 3). However, the magnitude of change was markedly different in the two fractions. In the first 2–3 weeks, the increases in light particle DBH far exceeded that of heavy particle DBH. At 1 and 2 weeks (days 21 and 28) after initiation of the highest dosage, the relative light/heavy distribution became normal even though the activities of each fraction were still markedly elevated.

Upon withdrawal, DBH in both fractions decreased to control levels within 10 days.

Morphine in concentrations up to 100 μM had no effect on the activities of TH, DBH, dopa-decarboxylase or monoamine oxidase *in vitro* from untreated animals.

Effects of morphine on uptake of epinephrine and metaraminol. The morphine-induced alterations in CA and DBH suggested that there might be changes in the number of functional secretory vesicles in the adrenal medulla. Therefore, the ability of isolated vesicles to incorporate exogenous amines was determined (Fig. 4). In general, the uptakes per gland paralleled the decreases and subsequent increases in catecholamine levels; however, the pattern of change of uptake of metaraminol *vis-à-vis* epinephrine appeared to be different in that increases in metaraminol uptake tended to exceed those in epinephrine uptake and CA content throughout the first 3 weeks of morphine administration. Upon continued administration of 100 mg/kg, this preferential change disappeared (day 28) and the uptakes per gland of both amines were increased over control levels to a lesser extent than were CA levels. Upon withdrawal, there was only a small change in the uptakes during the first 24 hours (day 29), although CA dropped markedly. Uptakes per gland and CA levels again reached a plateau between 3 and 5 days post-withdrawal. In this group of rats, the parameters were still somewhat elevated above control at 10 days after discontinuing morphine (day 38); however, the differences between increases in CA levels and uptakes per gland were not as marked as during the earlier withdrawal periods.

The uptake per 100 μg of CA is a measure of the abilities of vesicles to take up amines relative to their endogenous CA levels and therefore is dependent upon the uptake properties of individual vesicles (but not on the number of vesicles). As shown in Fig. 5, the patterns of incorporation of metaraminol and epinephrine were distinctly different. At 10 mg/kg there was little change in epinephrine uptake/100 μg of CA, but metaraminol uptake per 100 μg of CA appeared to be increased. At subsequent times with increased doses, epinephrine uptake per 100 μg of CA was decreased below control levels and remained low throughout

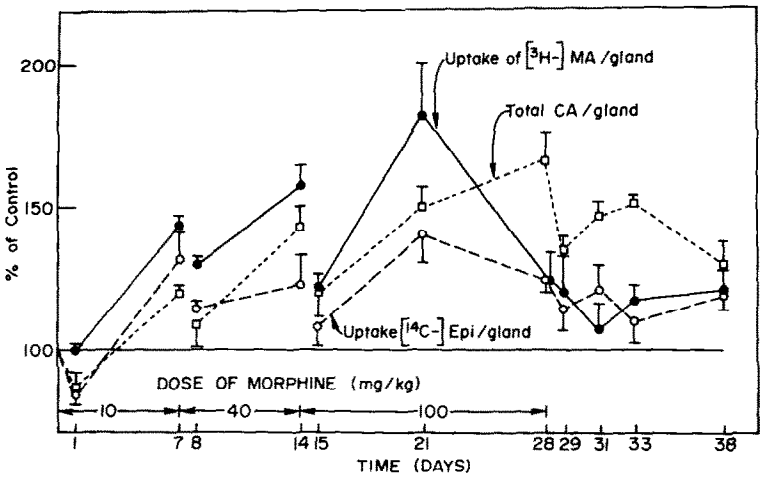

Fig. 4. Effects of morphine administration and withdrawal on uptakes per gland of epinephrine (Epi) and metaraminol (MA). Points and bars represent means ± standard errors of 5–11 animals. Control values (40 animals) were: epinephrine uptake, 4·07 ± 0·16 nmoles/gland; metaraminol uptake, 0·53 ± 0·02 nmoles/gland.

subsequent addiction and withdrawal, indicating impaired vesicle function. In contrast, metaraminol uptake per 100 μg of CA remained at or above control levels until 2 weeks at 100 mg/kg of morphine (day 28), at which point its uptake was also reduced; during subsequent withdrawal, metaraminol uptake per 100 μg of CA paralleled the decrease observed for epinephrine uptake.

Morphine in concentrations up to 100 μM had little or no effect *in vitro* on epinephrine and metaraminol uptakes in vesicles from untreated rats.

Effects of morphine on catecholamine efflux (*Table* 2). After 2 weeks at 100 mg/kg of morphine, the rate of efflux of catecholamines from isolated storage vesicles incubated in isotonic sucrose was reduced significantly compared to controls. Under these conditions, efflux represents outward leakage of amines across an intact vesicle membrane, rather than active release or lysis of vesicles [21]. Morphine in concentrations up to 100 μM had no effect *in vitro* on the effluxes of endogenous CA or newly incorporated [^{3}H]epinephrine from vesicles of untreated rats.

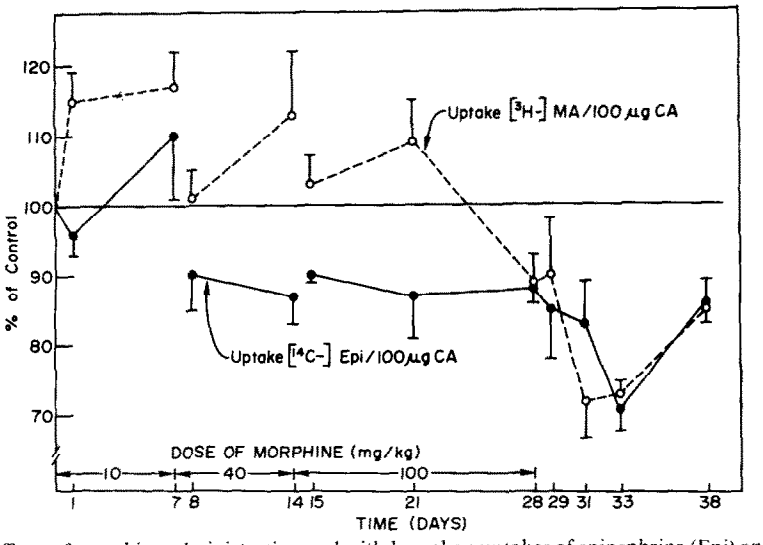

Fig. 5. Effects of morphine administration and withdrawal on uptakes of epinephrine (Epi) and metaraminol (MA) per 100 μg of endogenous catecholamines. Points and bars represent means ± standard errors of 5–11 animals. Control values (40 animals) were: epinephrine uptake, 30·6 ± 0·7 nmoles/100 μg of CA; metaraminol uptake, 3·97 ± 0·14 nmoles/100 μg of CA. Data are calculated from Fig. 4.

Table 2. Efflux of catecholamines from storage vesicles of control and morphine-treated rats*

Dose of morphine (mg/kg)	Percentage of catecholamines remaining in vesicles		T of late efflux phase (min)
	10-min Incubation	60-min Incubation	
0	93·4 ± 1·1	70·1 ± 1·0	120
100 (2 weeks)	97·4 ± 0·7†	81·6 ± 0·8‡	200

* Data represent means ± standard errors of three determinations at each time point. Initial values for catecholamine contents of the 26,000 *g* supernatants and pellets were: controls, 1·98 ± 0·02 μg/gland and 10·4 ± 0·5 μg/gland; morphine-treated, 1·89 ± 0·19 μg/gland and 20·8 ± 1·0 μg/gland.

† $P < 0·05$ vs control.

‡ $P < 0·001$ vs control.

Effects of morphine on insulin-induced secretion. The hypoglycemia which accompanies the administration of insulin produces a massive sympatho-adrenal discharge which can be used to determine whether neurogenic secretory function is normal. In rats given 100 mg/kg of morphine for 2 weeks, the degree of depletion upon subsequent insulin administration was equivalent to that in control rats (60 per cent depletion within 3 hr), indicating that the morphine-induced changes in adrenal medullary function do not alter the ability to respond to splanchnic nerve stimulation.

DISCUSSION

The acute administration of morphine results in partial depletion of adrenal medullary catecholamines, an effect which appears to be mediated by a central reflex mechanism [1–12]. Consequently, the depletion can be prevented by barbiturate administration [29], by peripheral blockade of splanchnic outflow or by splanchnicotomy [30, 31]. It has been noted that the morphine-induced catecholamine depletion is no longer evident after chronic administration and that the levels could even exceed those of controls [11, 12]; similar changes are found in central adrenergic neurons [8, 13]. It therefore was suggested that the development of tolerance to the analgesic effect of morphine was accompanied by tolerance to the reflex sympatho-adrenal discharge [32]. In the current study, we have obtained biochemical evidence which does not support this hypothesis.

Neurogenic secretion from the adrenal medulla occurs via exocytotic, all-or-none release of the vesicle contents, wherein soluble components (catecholamines, ATP, chromogranins, soluble DBH) are quantally extruded while the vesicle membranes (particulate DBH) remain behind [17, 33, 34]. The acute effects of morphine seen with each dosage increment are consistent with the view that the drug elicits splanchnic stimulation: there is a decrease in catecholamine levels accompanied by a parallel loss of ATP and usually of DBH, as well as a decline in the number of functional vesicles (as determined by the ability to incorporate [^{14}C]epinephrine). There is also a shift toward an increase in broken vesicle membranes which remain after secretion, as indicated by increases in DBH in the light particle fraction and the decrease in CA/DBH ratio in the whole gland.

Stimulation of the adrenal medulla is known to bring about a series of biochemical changes which accelerate the rate of recovery from depletion; these include induction of the rate-limiting enzyme, tyrosine hydroxylase [35–41], induction of dopamine β-hydroxylase [20, 35, 42] and increased synthesis of new storage vesicles [17, 20, 33]. Upon cessation of stimulation, these parameters usually return to normal within 4–10 days [20, 36]. In the current studies, chronic morphine administration produced a dose-dependent induction of both tyrosine hydroxylase and dopamine β-hydroxylase which persisted throughout the 28 days of treatment (2 weeks at the highest dose). When morphine was discontinued, activities of both enzymes declined to normal within 10 days. These data indicate that the sympatho-adrenal axis maintains a high level of stimulation throughout the course of morphine treatment, and that tolerance to this reflex does not develop. Therefore, the recovery of and subsequent increase in catecholamine levels upon chronic administration probably result from the development of a catecholamine synthetic capability which equals or exceeds the rate of secretion. In addition, there appears to be an increase in the storage capacity of the tissue, e.g. an increase in the number of storage vesicles which can accommodate the increased synthesis. This is reflected by the increase in the number of "immature" vesicles, typified by the shift in DBH distribution toward lower density particles, the increase in amine uptake per gland and the decreased preference for uptake of epinephrine versus metaraminol [17, 20]. It is interesting to note that by the second week of treatment with 100 mg/kg of morphine, the vesicle population appears to be "normal" in regard to the light/heavy distribution of DBH and to the relative uptakes of the two amines; while this may suggest that the reloading of vesicles with catecholamines has caught up to the rate of new vesicle synthesis, it should not be overlooked that other properties of the vesicles (fragility, efflux, uptake per 100 μg of CA) remain altered at

this time. In any case, the high levels of catecholamines, ATP and storage vesicles confirm that neurogenic input is elevated throughout the period of morphine administration.

Withdrawal from morphine addiction resulted in a multiphasic disappearance of the elevation in catecholamines, with an initial rapid decline (0–3 days) and subsequently a slower decline to control levels (5–10 days). Formerly, the initial loss has been attributed to neurogenic secretion due to withdrawal stress [8, 12, 13, 32, 43]. The present data indicate clearly that tyrosine hydroxylase, which increases upon neural stimulation, declines during all phases of withdrawal, suggesting that in fact neurogenic input decreases when morphine is discontinued. There is additional evidence that increased secretion is not taking place during withdrawal: there is no change in the subcellular distribution of DBH (the light/heavy ratio should rise during acutely increased stimulation), and there is no change in the preference for uptake of epinephrine versus metaraminol (the epinephrine/metaraminol ratio should fall during increased stimulation). While it is possible that a non-neurogenic, non-exocytotic secretion takes place (for example, increased leakage of catecholamines from vesicle to cytoplasm and from cytoplasm to extracellular space), this explanation seems unlikely, and the present data suggest an altogether different explanation for the rapid and slow phases of decline in CA, i.e. chronic morphine administration may lead to the formation of a "defective" population of vesicles with a slow turnover rate relative to that of the normal population. One indication that this may occur is that during the initial withdrawal period, while enzyme activities and catecholamines decline toward control levels, the decreased uptake per 100 μg of CA actually worsens. In a "normal" vesicle population, 3 days is a reasonable turnover time in a post-stimulation period [20]. However, if the vesicles with a defective uptake system have a slower turnover, then when morphine is discontinued, the number of "normal" vesicles will decline more rapidly than will the defective ones, resulting in two different rates of decline in CA and DBH and an initial worsening of the uptake per 100 μg of CA.

The morphine-induced defect in uptake cannot be accounted for by changes in intravesicular storage, since efflux and fragility were decreased by morphine. Rather, the data suggest that there is a decrease in inward transport, i.e. an effect involving the amine carrier in the vesicle membrane [44–50]. This could explain why epinephrine uptake per 100 μg of CA is affected considerably before metaraminol uptake, as the two amines use different membrane transport systems [21, 51–53]. The changes in fragility and efflux may also result from an alteration in the vesicle membrane [54]. The present data do not determine whether these effects result from prolonged neural stimulation of the tissue or from long-term exposure to morphine itself. Yoshizaki [55] has demonstrated a small, direct catecholamine-releasing effect of morphine in denervated rat adrenals, and similar studies should be done with chronically treated animals to determine which effects of morphine on the vesicles are neural in origin and which are directly attributable to the drug.

The adrenal medulla has often been used as a model of adrenergic neurons because of the morphological, biochemical, embryological and functional similarities. Other investigators [14] have found a lack of development of tolerance to the morphine induced stimulation of catecholamine synthesis in rat brain, although different results have been obtained in other species [15]. The results obtained in the adrenal suggest that morphine might induce a wider variety of changes in the synthesis, uptake, storage and release of brain catecholamines than has been heretofore identified.

In conclusion these data indicate that in rats: (1) tolerance to the sympatho-adrenal reflex elicited by morphine does not develop; (2) the re-establishment of catecholamine stores results from increased synthesis of amines and vesicles; and (3) chronic morphine administration induces a change in the functional properties of the storage vesicles.

Acknowledgement—The authors thank Mr. Frederic J. Seidler for his technical assistance.

REFERENCES

1. T. R. Elliott, *J. Physiol., Lond.* **44,** 374 (1912).
2. G. N. Stewart and J. M. Rogoff, *J. Pharmac. exp. Ther.* **19,** 59 (1922).
3. T. Hayama, *Jap. J. med. Sci.* **4,** 41 (1932).
4. H. Sato and F. Ohmi, *Tohoku J. exp. Med.* **21,** 411 (1933).
5. N. Emmelin and R. Stromblad, *Acta physiol. scand.* **24,** 260 (1951).
6. A. S. Outschoorn *Br. J. Pharmac. Chemother.* **1,** 605 (1952).
7. Y. Satake, *Tohoku J. exp. Med.* **60** (suppl 2), 1 (1954).
8. E. W. Maynert and G. I. Klingman, *J. Pharmac. exp. Ther.* **135,** 285 (1962).
9. L.-M. Gunne, *Nature, Lond.* **195,** 815 (1962).
10. T. B. B. Crawford and W. Law, *Br. J. Pharmac. Chemother.* **13,** 35 (1958).
11. T. Abe, *Jap. J. med. Sci.* **4,** 100 (1929).
12. E. W. Maynert, in *The Addictive State* (Ed. A. Wikler), p. 89. Williams & Wilkins, Baltimore (1968).
13. L.-M. Gunne, *Nature, Lond.* **184,** 1950 (1959).
14. D. H. Clouet and M. Ratner, *Science, N.Y.* **168,** 854 (1970).
15. C. B. Smith, J. E. Villareal, J. H. Bednarczyk and M. I. Sheldon, *Science, N.Y.* **170,** 1106 (1970).
16. E. L. Way and F. H. Shen, in *Narcotic Drugs: Biochemical Pharmacology* (Ed. D. Clouet), p. 229. Plenum, New York (1971).
17. T. A. Slotkin and N. Kirshner, *Biochem. Pharmac.* **22,** 205 (1973).
18. A. D. Smith and H. Winkler, *Biochem. J.* **103,** 480 (1967).
19. T. A. Slotkin and K. Edwards, *Biochem. Pharmac.* **22,** 549 (1973).
20. T. A. Slotkin and N. Kirshner, *Molec. Pharmac.* **9,** 105 (1973).
21. T. A. Slotkin, R. M. Ferris and N. Kirshner, *Molec. Pharmac.* **7,** 308 (1971).

22. J. C. Waymire, R. Bjur and N. Weiner, *Analyt. Biochem.* **43,** 588 (1971).
23. S. Friedman and S. Kaufman, *J. biol. Chem.* **240,** 4763 (1965).
24. D. S. Duch, O. H. Viveros and N. Kirshner, *Biochem. Pharmac.* **17,** 255 (1968).
25. P. Laduron and F. Belpaire, *Biochem. Pharmac.* **17,** 1127 (1968).
26. R. J. Merrills, *Analyt. Biochem.* **6,** 272 (1963).
27. B. L. Strehler and J. K. Totter, in *Methods of Biochemical Analysis* (Ed. D. Glick), Vol. 1, p. 344. Interscience, New York (1954).
28. R. L. Wine, *Statistics for Scientists and Engineers*, p. 250. Prentice-Hall, Englewood Cliffs, N.J. (1964).
29. E. W. Maynert, *J. Pharmac. exp. Ther.* **143,** 90 (1964).
30. G. N. Stewart and J. M. Rogoff, *J. exp. Med.* **24,** 709 (1916).
31. H. Sibuta, K. Endo and G. Nagakura, *Tohoku J. exp. Med.* **50,** 1 (1949).
32. L.-M. Gunne, *Acta physiol. scand.* **58** (suppl. 204), 1 (1963).
33. O. H. Viveros, L. Arqueros and N. Kirshner, *Molec. Pharmac.* **7,** 444 (1971).
34. O. H. Viveros, L. Arqueros and N. Kirshner, *Science, N.Y.* **165,** 911 (1969).
35. O. H. Viveros, L. Arqueros, R. J. Connett and N. Kirshner, *Molec. Pharmac.* **5,** 69 (1969).
36. R. L. Patrick and N. Kirshner, *Molec. Pharmac.* **7,** 87 (1971).
37. N. Weiner and W. F. Mosimann, *Biochem. Pharmac.* **19,** 1189 (1970).
38. R. A. Mueller, H. Thoenen and J. Axelrod, *J. Pharmac. exp. Ther.* **169,** 74 (1969).
39. H. Thoenen, R. A. Mueller and J. Axelrod, *Nature, Lond.* **221,** 1264 (1969).
40. V. DeQuattro, R. Maronde, T. Nagatsu and N. Alexander, *Fedn. Proc.* **27,** 1240 (1968).
41. R. Kyetňansky, V. K. Weise and I. J. Kopin, *Endocrinology* **87,** 744 (1970).
42. P. B. Molinoff, S. Brimijoin, R. Weinshilboum and J. Axelrod, *Proc. natn. Acad. Sci. U.S.A.* **66,** 453 (1970).
43. Y. Tachigawa, *J. orient. Med.* **17,** 521 (1932).
44. G. Taugner, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **270,** 392 (1971).
45. G. Taugner, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **274,** 299 (1972).
46. B. Agostini and G. Taugner, *Histochemie* **30,** 255 (1973).
47. A. Pletscher, M. DaPrada, H. Steffen, K. H. Berneis and B. Lütold, *Third Int. Catecholamine Symp.* (1973).
48. T. A. Slotkin, *Life Sci.* **13,** 675 (1973).
49. T. A. Slotkin and N. Kirshner, *Biochem. Pharmac.* **22,** 2492 (1973).
50. T. A. Slotkin, *Biochem. Pharmac.* **24,** 89 (1975).
51. T. A. Slotkin and N. Kirshner, *Molec. Pharmac.* **7,** 581 (1971).
52. P. Lundborg, *Acta physiol. scand.* **67,** 423 (1966).
53. P. Lundborg and R. Stitzel, *Br. J. Pharmac. Chemother.* **29,** 42 (1967).
54. T. A. Slotkin and H. O. Green, *Biochem. Pharmac.* **23,** 2190 (1974).
55. T. Yoshizaki, *Jap. J. Pharmac.* **23,** 695 (1973).