NARCOTIC ANALGESICS AND THE REGULATION OF NEURONAL CATECHOLAMINE STORES

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INTRODUCTION

SEVERAL putative central neurotransmitters (MAYNERT, 1967; WAY *et al.*, 1968; CLOUET, 1971) including noradrenaline and dopamine (CLOUET and RATNER, 1970; SESAME *et al.*, 1972; SMITH *et al.*, 1972; LOH *et al.*, 1973) have been implicated in the pharmacological actions of morphine. CLOUET and RATNER (1970) measured radioactive 3,4-dihydroxyphenylalanine (DOPA) norepinephrine (NE) and dopamine (DA) in various brain parts of rats receiving acutely (1 or 2 hr before labeling) or chronically (1 dose per day for 5 days) 210 μmoles/kg of morphine. They found that morphine increases the conversion of ^{14}C tyrosine into catecholamines and that tolerance to this morphine effect fails to develop. In contrast a report by SMITH *et al.* (1972) shows that tolerance to the analgesic effects of morphine is associated with tolerance to the increase of catecholamine synthesis elicited by morphine. LOH *et al.* (1973) also performed a study of radioactive tyrosine conversion into NE and DA in the whole brain of mice. They used two doses of morphine (13 and 52 μmoles/kg) and estimated the turnover rate of brain catecholamines using the accumulation index which takes in account the specific activity of the precursor tyrosine. They found that although morphine (52 μmoles/kg s.c.) increases the conversion of radioactive tyrosine into brain DA and NE it does not significantly change the accumulation index. FRIEDLER *et al.* (1972) have reported that 6-hydroxydopamine (6OHDA) which causes a selective degeneration of the adrenergic neurons in the brain, reduces morphine analgesia, but it does not prevent the development of morphine tolerance. Moreover, the pretreatment with 6OHDA exacerbates the signs of morphine withdrawal.

ESTIMATION OF CATECHOLAMINE TURNOVER RATE: EFFECT OF ACUTE MORPHINE, CHRONIC MORPHINE, VIMINOL AND NALOXONE

We have estimated the turnover rate of cerebellar NE, spinal cord NE and striatal DM by injecting intravenously 1 mCi/kg of L-tyrosine 3,5-^{3}H (1 mCi/33 nmoles) and by measuring the specific radioactivity of tyrosine, NE or DA. We selected these brain areas, because each area contains only dopaminergic or noradrenergic axons. Drugs were injected i.p. 10 min before the radioactive tyrosine and the animals were killed 10 min after the label. Specific radioactivity of tyrosine, NE and DA and the turnover rate of these amines were determined as reported earlier (NEFF *et al.*, 1971; COSTA *et al.*, 1972). Animals were rendered tolerant to and physically dependent on morphine by pellet implantation (HO *et al.*, 1972). Physical dependence was assessed by the naloxone test (CHENEY *et al.*, 1972). The data reported in Table 1 show that morphine (52 μmoles/kg, i.p.) increases the turnover rate of DA in striatum,

TABLE 1. TURNOVER RATE OF NE AND DA IN BRAIN PARTS OF RATS RECEIVING VARIOUS DOSES OF MORPHINE

Morphine (μmoles/kg, i.p.)	Turnover rate (nmoles/g/hr ± S.E.)		
	Striatal DM	Cerebellar NE	Spinal cord NE
None	15 ± 1·7	0·45 ± 0·054	0·72 ± 0·074
13	22 ± 6·6	0·34 ± 0·053	0·81 ± 0·16
26	25 ± 6·4	0·47 ± 0·014	0·86 ± 0·12
[illegible]	24 ± 2·6*	0·50 ± 0·068	0·96 ± 0·093
Pellets (1950 s.c.)	18 ± 4·2	0·52 ± 0·067	0·64 ± 0·11

Each value represents the mean of four experiments. Pellets were implanted 72 hr before measuring the catecholamine turnover rate.
* $P < 0·02$

but it does not change the turnover rate of spinal cord and cerebellar NE. Moreover, in rats implanted with morphine pellets the turnover rate of striatal DA is normal. To study the tolerance to the increase of striatal DA turnover elicited by morphine we implanted rats with morphine pellets for 72 hr. Ten minutes before injecting the label, they received an additional dose of morphine intraperitoneally. The results of these experiments are shown in Table 2. An intraperitoneal injection of 52 μmoles/kg of morphine to rats implanted with morphine pellets no longer increases the turnover of striatal DA. The data reported in Table 2 also show that high doses of morphine injected to rats implanted with morphine pellets increase the striatal DA turnover rate but these doses increase the turnover rate of neither cerebellar nor spinal cord NE.

Using the tail flick test we found that the morphine dose that increases the turnover rate of striatal DA is an AD_{80} for analgesia in 20 min. We found that like the analgesia also the increase of striatal DA turnover rate exhibits tolerance. The relationship between morphine analgesia and DA turnover is corroborated by the data reported in Table 3. They concern some stereoisomers if viminol (1[α(N-0-chlorobenzyl) pyrryl] 2-di-sec butylamine ethanol) a central analgesic which exhibits cross tolerance to morphine (Della Bella *et al.*, 1973). The data listed in Table 3 show that the stereoisomer R_2 can elicit central analgesia and a dose AD_{80} for analgesia can

TABLE 2. TURNOVER RATE OF NE AND DA IN BRAIN PARTS OF RATS IMPLANTED WITH MORPHINE PELLETS AND RECEIVING VARIOUS DOSES OF MORPHINE

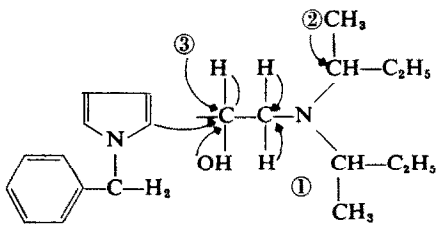
Morphine (μ moles /kg, i.p.)	Turnover rate (nmoles/g/hr ± S.E.)		
	Striatal DM	Cerebellar NE	Spinal cord NE
Pellets (1950 s.c.) + saline	18 ± 4·2	0·52 ± 0·063	0·64 ± 0·11
Pellets + 52	28 ± 2·7	0·69 ± 0·093	0·75 ± 0·068
Pellets + 208	32 ± 2·9*	0·52 ± 0·011	0·61 ± 0·072
Pellets + 416	48 ± 5·0†	0·68 ± 0·11	0·93 ± 0·16

Each value represents the mean of four experiments. Pellets were implanted 72 hr before injecting morphine
* $P < 0·05$
† $P < 0·005$

TABLE 3. TURNOVER RATE OF STRAITAL DA IN RATS RECEIVING VARIOUS DOSES OF VIMINOL**

Type of stereoisomer	μmoles/kg, i.p.	Analgesia AD	Turnover rate (nmoles/g/hr ± S.E.)
None	—	—	25 ± 1·8
R_2	1·7	20	22 ± 3·2
R_2	3·5	60	24 ± 2·9
R_2	7·0	80	48 ± 3·6*
R_2	14·0	90	54 ± 3·8*
S_2	56·0	0	26 ± 2·4
MESO	28	0	29 ± 3·0

Each value represents the mean of at least 4 experiments
* $P < 0{\cdot}001$



R_2 is C_1 = R; C_2 = R; C_3 = $([\alpha]_D 20°\Delta\ 19{\cdot}46)$
S_2 is C_1 = S; C_2 = S; C_3 = $-([\alpha]_D\ 20 + 1{\cdot}10)$

increase the turnover rate of striatal DA. In contrast the stereoisomer S_2 was injected in doses four times greater than those of R_2, but it neither increased the turnover rate of striatal DA nor did it cause analgesia. Similarly the Meso isomers, which are weak analgesics, did not increase the turnover rate of striatal DA when injected in doses devoid of analgesic activity (Table 3).

However, the relationship between increase of striatal DA turnover rate and analgesia failed to be completely supported when it was tested using naloxone. This pure morphine antagonist which is devoid of analgesic activity was injected intraperitoneally in various doses, it increased the turnover rate of striatal DA and cerebellar NE when given in high doses (Table 4).

TABLE 4. TURNOVER RATE OF NE AND DA IN BRAIN PARTS OF RATS INJECTED WITH VARIOUS DOSES OF NALOXONE

Naloxone (μmoles/kg, i.p.)	Turnover rate (nmoles/g/hr ± S.E.)	
	Striatal DN	Cerebellar NE
None	17 ± 2·7	0·65 ± 0·043
22	22 ± 2·9	0·62 ± 0·052
44	20 ± 2·8	0·69 ± 0·014
88	29 ± 2·4*	0·83 ± 0·091†

Each value represents the mean of four experiments.
* $P < 0{\cdot}01$
† $P < 0{\cdot}05$

INTERPRETATION OF THE MORPHINE ACTION ON STRIATAL DA TURNOVER RATE

Current understanding of the relationship between drug effects on striatal DA turnover rate and their ability to cause an indirect stimulation of dopaminergic receptors is far from perfect. In fact, amphetamine which indirectly stimulates dopaminergic receptors (COSTA *et al.*, 1972) and chlorpromazine which blocks DA receptors (NEFF and COSTA, 1966) increase the turnover rate of striatal DA. A morphine dose twice that increasing the turnover rate of striatal DA causes catalepsy, a postural plasticity with increased muscle tone, which resembles parkinsonian rigidity. This similarity would support the theory that morphine blocks doapminergic receptors, although, muscular rigidity can be elicited pharmacologically without involving directly or indirectly dopaminergic neuronal functions.

Evidence is now available indicating that striatum contains a dopamine sensitive adenylate cyclase (KEBABIAN *et al.*, 1972). This information suggested to us to investigate whether the dose of (+) amphetamine that selectively increases the turnover rate of striatal dopamine also increases the concentrations of cyclic 3',5'-adenosine monophosphate (cAMP) in striatum. These studies have become feasible with the availability of microwave sources that can be focused to allow inactivation of brain enzymes in 2 sec. Previously such studies were plagued by continuous post mortem changes of cAMP concentrations in brain (UZUNOV and WEISS, 1972).

The data of Table 5 show a dose dependent correlation between the increase of DA turnover rate, and of cAMP concentrations in striatum and the increase of motor activity elicited by (+) amphetamine. The data reported in Fig. 1 show that 104 and 260 μmoles/kg, i.p. of morphine increased the concentrations of cAMP but 52 μmoles/kg did not. The latter dose was threshold for increasing the turnover rate of striatal DA. We have analysed the content of cAMP at 30 min after morphine injection (104 μmoles/kg) in cerebellum and hypothalamus and found that the cAMP content of these brain areas was not increased. The data of Fig. 1 also show that 52 μmoles/kg, i.p. of morphine increase the concentrations of cAMP in pituitary, adrenal cortex and medulla. As shown in Fig. 2 (+) amphetamine increases cAMP concentrations in striatum, but fails to change the concentrations of cAMP in pituitary, adrenal cortex and medulla. The increase of cAMP in striatum, pituitary, adrenal medulla and cortex elicited by morphine lasts for several hours (Fig. 3). This long lasting increase of the cAMP is quite unusual, the cAMP increase elicited

TABLE 5. EFFECTS OF VARIOUS DOSES OF (+) AMPHETAMINE ON MOTOR ACTIVITY, TURNOVER RATE OF TELENCEPHALIC NE, STRIATAL DA AND STRIATAL CONCENTRATIONS OF cAMP

(+) amphetamine (μmoles/kg, i.p.)	Motor activity (events/min ± SE)	Telencephalic NE (nmoles/g/hr ± SE)	Striatal DA (nmoles/g/hr ± SE)	Striatal cAMP (pmoles/mg protein)
NO	4·5 ± 1·7	2·1 ± 0·31	28 ± 3·2	2·8 ± 0·20
0·4	2·5 ± 0·71	1·9 ± 0·27	24 ± 2·9	4·2 ± 0·74
3·2	25 ± 7·4*	2·3 ± 0·41	67 ± 7·2*	9·2 ± 1·8*

* $P < 0{\cdot}01$

Each value is the average of at least four measurements. Motor activity was measured as described by COSTA *et al.* (1972).

Striatal cAMP concentrations, motor activity and monamine turnover rate were measured at 15 min post injection.

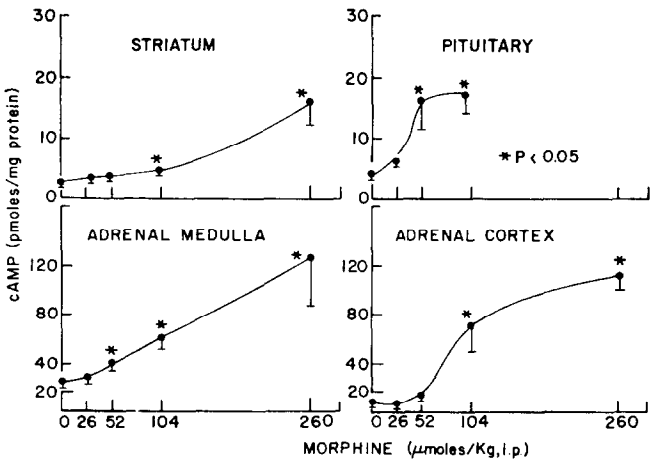


FIG. 1.—Concentrations of cAMP in striatum, pituitary adrenal cortex and medulla thirty minutes after increasing doses of morphine. cAMP concentrations were measured with a modification of the method reported by EBADI *et al.* (1971).

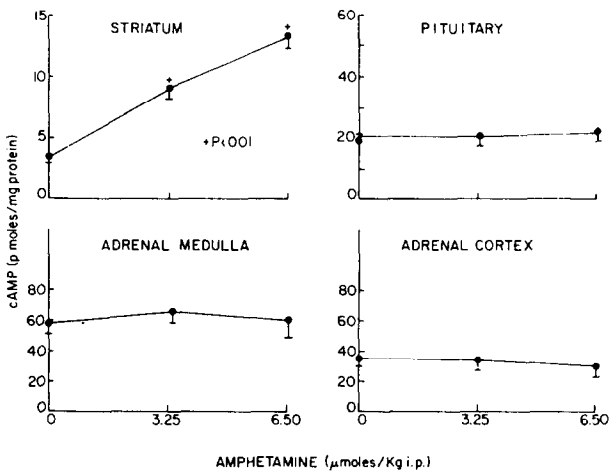


FIG. 2.—Concentrations of cAMP in striatum, pituitary, adrenal cortex and medulla fifteen minutes after increasing doses of (+) amphetamine.

by various drugs and by exposure of the rat to 4°C (GUIDOTTI and COSTA, 1973; GUIDOTTI *et al.*, 1973) does not last longer than 2 hr. Preliminary data suggest that the increase of striatal cAMP elicited by (+) amphetamine does not last longer than 1 hr.

EFFECTS OF MORPHINE ON cAMP CONCENTRATIONS AND TYROSINE HYDROXYLASE ACTIVITY OF ADRENAL MEDULLA

The data of Fig. 4 show that the cAMP concentration in adrenal cortex and medulla of rats implanted with morphine pellets is equal to that of rats sham operated. Moreover, when sham operated or morphine pellets implanted rats are exposed to 4°C for 30 min the increase of cAMP is greater in morphine pellets implanted rats than in rats receiving saline. This greater increase of cAMP concentration can be seen in both cortex and adrenal medulla.

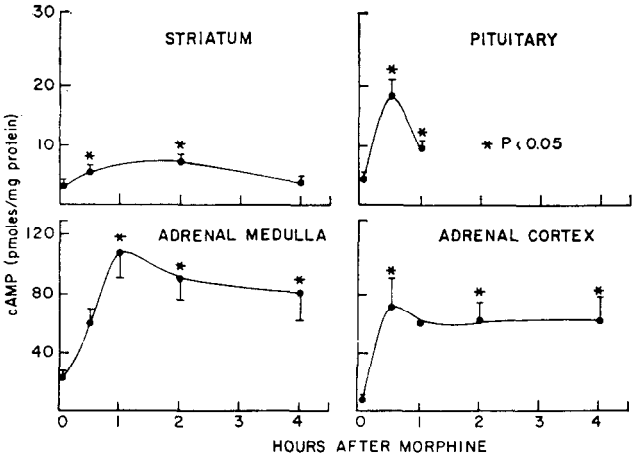


FIG. 3.—Time course of the increase of cAMP concentrations elicited by 104 μmoles/kg, i.p. of morphine in striatum, pituitary adrenal medulla and cortex.

The data reported in Figs. 1 and 2 show that morphine (52 μmoles/kg, i.p.) increases the concentrations of cAMP in medulla whereas (+) amphetamine fails to increase the cAMP concentration in this chromaffine tissue. We have shown that a number of drugs that increase cAMP concentrations in medulla increase tyrosine hydroxylase activity after a latency time of 10–14 hr (GUIDOTTI and COSTA, 1973). Morphine is not an exception to this sequence of events. As shown in Fig. 5, 24 hr after a morphine injection (52 μmoles/kg, i.p.) tyrosine hydroxylase activity is increased in intact adrenal glands but fails to increase in denervated adrenals. Moreover, the data reported in Fig. 5 show that rats implanted with morphine pellets fail to exhibit an increase of tyrosine hydroxylase activity. We have measured tyrosine hydroxylase activity in the hypothalamus, striatum and cerebellum of these rats and found that the activity of this enzyme is not increased.

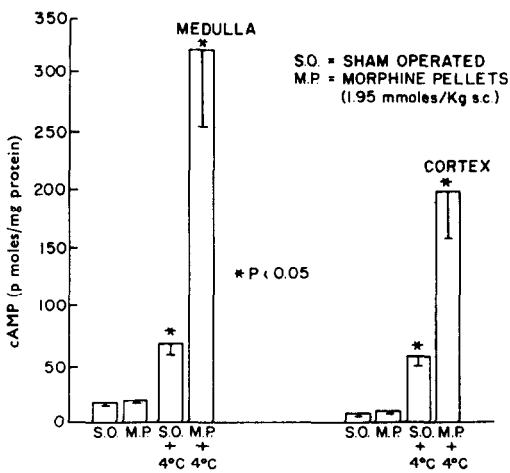


FIG. 4.—cAMP concentrations in adrenal cortex of rats implanted with morphine pellets (1·95 mmoles/kg, s.c.) or sham implanted. Both groups of rats were either kept at 24°C or at 4°C for 30 min.

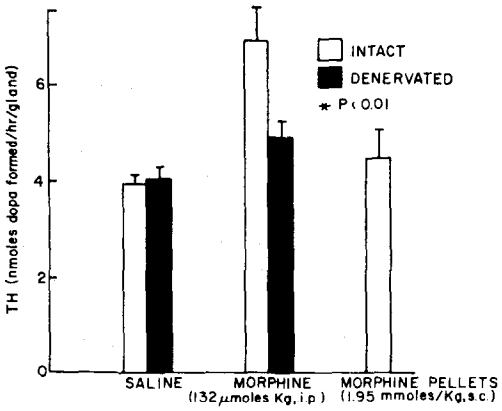


FIG. 5.—Tyrosine hydroxylase activity in intact and denervated (5 days before) adrenal glands of rats injected with either 132 μmoles/kg, i.p. of morphine, or morphine pellets (1·95 mmoles/kg, s.c.). Enzyme activity was measured according to WAYMIRE *et al.* (1971).

CONCLUSION

The data presented suggest the following:

(1) Doses of morphine and viminol which are very close to the AD_{80} for analgesia, increase the turnover rate of striatal DA. The turnover rate of NE in spinal cord and cerebellum of these animals is not increased.

(2) Like analgesia, the increase of DA turnover elicited by morphine shows tolerance.

(3) Naloxone, a pure morphine antagonist, increases turnover rate of DA and cerebellar NE when injected in very high doses (88 μmoles/kg, i.p.).

(4) (+) amphetamine like morphine, selectively stimulates the turnover rate of striatal DA. Both drugs increase the concentrations of cAMP in striatum. However, the effect of morphine persists longer than that of amphetamine, in addition, unlike amphetamine the action of morphine on cAMP does not strictly correlate to its action on DA turnover rate.

(5) If the increase of cAMP in striatum reflects a stimulation of postsynaptic dopaminergic receptors, judging from the persistence of this response, it might be inferred that morphine stimulates persistently, these receptors. The location of the morphine action on cAMP concentrations and DA turnover rate to the striatum is of interest in view of reports that this brain structure contains the highest concentration of opiate receptors among various brain areas studied (PERT and SNYDER, 1973).

(6) Morphine injections promptly increase cAMP concentration in adrenal medulla and stimulate tyrosine hydroxylase activity of this tissue after a time delay of almost 24 hr. This increase of enzyme activity, like the increase of cAMP, requires the presence of cholinergic nerves suggesting that morphine does not increase cAMP because it blocks phosphodiesterase (like aminophyllin COSTA and GUIDOTTI, 1973) or because it stimulates medullary cholinergic receptors (like carbamylcholine COSTA and GUIDOTTI, 1973).

(7) The action of morphine on cAMP concentrations in adrenal medulla and cortex shows tolerance. However, rats tolerant to the increase of cAMP elicited by morphine are still responsive to the increase of cAMP elicited by cold exposure (4°C).

SUMMARY

Morphine (52 μmoles/kg, i.p.) increases the turnover rate of DA in striatum. Quantitatively, this action appears related to the analgesic effect but not to an increase of cAMP concentration of striatum which requires 104 μmoles/kg, i.p. The action of morphine on DA turnover shows tolerance. (+) amphetamine doses that enhance the turnover of striatal DA also increase cAMP concentrations in striatum. While the increase of striatal cAMP elicited by threshold doses of morphine lasts longer than two hours that of (+) amphetamine lasts less than one hour. An identity of the mode of action of morphine and (+) amphetamine on striatal dopaminergic functions was not established. Morphine injections promptly increase cAMP concentration in adrenal medulla and subsequently increase tyrosine hydroxylase activity. Both effects show tolerance, and require intact cholinergic innervation.

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