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EFFECTS OF MORPHINE ON THE RAT STRIATAL DOPAMINE METABOLISM

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Abstract—Concomitant with total suppression of the spontaneous unitary discharges of neurons in the rat corpus striatum, intracarotidly injected morphine (5 mg/kg) was also found to increase the levels of dopamine, homovanillic acid and cyclic AMP by 80, 65 and 46 per cent respectively, measured 5 min after injection. This provides further support to the hypothesis that the nigrostriatal dopaminergic pathway is stimulated by acutely administered morphine. Morphine (10^{-5}–10^{-3} M) did not alter the activity of striatal tyrosine aminotransferase. The drug, added *in vitro* (10^{-6}–10^{-4} M) or by intracarotid injection (5 mg/kg) did not affect the activity of striatal tyrosine hydroxylase. Moreover, morphine (10^{-4} M) did not interfere with the inhibitory effects of dopamine (10^{-6}–10^{-4} M) on striatal tyrosine hydroxylase. However, it significantly potentiated the stimulatory effects of cyclic AMP on this enzyme. Morphine (10^{-5}–10^{-4}) was also found to have no effect on the spontaneous or K^+-stimulated release of dopamine from striatal homogenate and synaptosomes. However, in the presence of 5×10^{-5} M and 10^{-4} M morphine, the uptake of dopamine by striatal homogenate was inhibited by 14 and 33 per cent respectively. With synaptosomal preparations, dopamine uptake was inhibited by 17 per cent in the presence of 10^{-4} M morphine—the inhibition being competitive with dopamine with an apparent K_i of 0.41 mM. The inhibition of dopamine uptake caused by 10^{-4} M morphine in either preparation was not reversed by the addition of 10^{-4} M naloxone. It was concluded that the increase in dopaminergic activity following acute treatment of morphine is probably due to (1) prolongation of the effect of dopamine on the post-synaptic neurons resulting in increased production of cyclic AMP which in turn potentiates dopamine synthesis and (2) decrease in presynaptic cystosol dopamine which is normally a feedback inhibitor of tyrosine hydroxylase thus leading to increased synthesis of dopamine.

Morphine, injected intracarotidly into rats, was found to inhibit almost instantaneously the spontaneous unitary discharges of neurons in the corpus striatum in a pharmacologically specific manner [1–3]. This inhibition was probably due to an increase in dopaminergic activities in the nigrostriatal pathway, since that it could be prevented by dopamine receptor blockers [2, 3]. If this is true, the inhibitory effect should be associated with concomitant rises in dopamine and its metabolites. Administration of morphine, in general, has been shown to increase the synthesis and turnover of dopamine in mammalian whole brains and particularly in the corpus striatum [4]. However, the mechanism underlying this stimulatory action of morphine is presently unknown.

The synthesis of dopamine begins with the *meta*-hydroxylation of cytoplasmic L-tyrosine by tyrosine hydroxylase (tyrosine 3-monooxygenase, EC 1.14.16.2) which is followed by decarboxylation by the action of aromatic amino acid decarboxylase. The level of free dopamine in the intraneuronal cytoplasm regulates the rate of dopamine synthesis by a negative feedback effect on tyrosine hydroxylase. This mechanism is thought to be most critically involved in the regulation of catecholamine synthesis in the brain [5–8]. Another possible regulatory mechanism is the availability of precursor tyrosine for dopamine synthesis. Tyrosine may undergo transamination with 2-oxoglutarate by tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5). Hence, transamination may compete with the catecholamine biosynthetic pathway for the pool of tyrosine [9, 10]. The dopamine synthesized is stored in vesicles and is released into the synaptic cleft during nerve impulse transmission [11]. Termination of the action of dopamine is mainly the result of active re-uptake of the transmitter by presynaptic neurons [12], and to some extent by diffusion and enzymatic degradation.

An increase in dopaminergic activity upon morphine administration may, therefore, result from an increase in the activities of the dopamine biosynthetic pathway, an increase in dopamine release or a decrease in dopamine re-uptake. The following studies were made to investigate whether these processes are involved in the neurochemical action of morphine in the corpus striatum of rats.

MATERIALS AND METHODS

Materials. L-[U-^{14}C]Tyrosine hydrochloride (10 mCi/m-mol), [ring-G-^{3}H]dopamine (8.5 Ci/m-mol) and cyclic AMP assay kits were purchased from the Radiochemical Centre, Amersham, U.K. Morphine sulphate was obtained from May and Baker. Naloxone

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was from Endo Lab. ATP, 2-oxoglutarate, cyclic AMP, L-tyrosine, L-dopamine, homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid), Tris buffer, Triton X-100, L-β-3,4 dihydroxyphenylalanine (L-DOPA) and bovine serum albumin were all products of Sigma Chemical Co., U.S.A. 2-Amino-4-hydroxy-6,7-dimethyltetrahydropteridine ($DMPH_4$) was a gift from Dr. J. H. Tong, University of Ottawa, Canada. Other chemicals were of analytical grade from various companies.

Measurements of dopamine, homovanillic acid and cyclic AMP. Male adult Sprague–Dawley rats weighing between 150–200 g were used. All experiments were performed between 9.30 and 10.00 a.m. Cannulation of the left carotid artery, centrifugal to the heart, was performed under pentobarbital anaesthesia (50 mg/kg, i.p.). Morphine (5 mg/kg) or the same volume of 0.9% saline was injected intracarotidly. The animal was decapitated after 5 min and the corpus striatum was dissected out immediately on an ice block. A transverse section was made at the caudal level of the olfactory bulb to expose the caudate heads. This was followed by a second transverse section at the caudal level of the optic chiasma. Then the corpus striatum from both hemispheres was dissected out with the lateral ventricles and corpus callosum as internal and external limits.

Striatal tissues from each animal were pooled and homogenised in ice cold 0.42 M perchloric acid. The contents of dopamine [13], homovanillic acid [14] and cyclic AMP [15] in the neutralized, deproteinized extracts were determined. Morphine interfered with the fluorimetric assays and was separated from dopamine on Dowex-50 (H^+ form) column and from homovanillic acid by extraction with butylacetate. The results were corrected for recovery by the values: dopamine 68 ± 4% (mean ± S.E.M., $n = 15$) and homovanillic acid 58 ± 2% (mean ± S.E.M., $n = 16$).

Measurement of tyrosine aminotransferase (TAT) activity. Striatal tissues from 2–4 animals were pooled and homogenized in 10 volumes (w/v) of chilled 0.1 M potassium phosphate buffer (pH 7.4) containing 1% Triton X-100. The clear supernatant obtained by centrifugation at 20,000 *g* for 30 min at 4° was used as a source of TAT. The activity of TAT was measured spectrophotometrically [16]. The assay contained, in 1 ml, 100 μmol potassium phosphate buffer (pH 7.4), 4 μmol L-tyrosine, 20 μmol 2-oxoglutarate, 1 μmol EDTA, 4 μmol diethyldithiocarbamate and various amounts of morphine if tested. After preincubation for 1 min at 37°, the reaction was started by the addition of the enzyme preparation (about 0.7 mg of protein) and allowed to proceed for 15 min. The reaction was stopped by mixing with 0.2 ml of 10 N KOH and left at room temperature for 30 min. Then, 0.2 ml of absolute ethanol was added and the absorbance at 331 nm was read in a Varian Techtron VS-VIS spectrophotometer. An extinction coefficient of 19900 M^{-1} was used to calculate the amount of *p*-hydroxyphenylpyruvate formed [16]. Blank activity was measured in an incubation mixture to which KOH was added prior to the addition of enzyme. The enzyme activities, expressed as μmol of *p*-hydroxyphenylpyruvate formed/hr/g fresh weight of tissue were means of duplicated assays.

Measurement of tyrosine hydroxylase (TH) activity. The left carotid artery of the rat was cannulated for morphine (5 mg/kg) or saline (0.9%) injection. The animal was decapitated 5 min after injection and the corpus striatum dissected out as described before. In some cases, controls were used in which striatal tissues were obtained from normal animals without surgical operations. Tissues from 2–4 animals were pooled and homogenized in 5 volumes (w/v) of ice cold 0.05 M Tris–HCl buffer (pH 6) containing 0.2% Triton X-100. The homogenate was centrifuged at 10,000 *g* at 4° for 10 min and the activity of tyrosine hydroxylase in the clear supernatant was determined [17].

The assay mixture contained, in a final volume of 1 ml, 0.01 μmol L-[U-^{14}C]tyrosine (4.06 mCi/m-mol), $FeSO_4.7H_2O$, 0.1 μmol $DMPH_4$, 125 μmol 2-mercaptoethanol, 200 μmol sodium acetate (pH 6) and when applicable, varying amounts of test substances (morphine, cyclic AMP, Mg^{2+}, ATP and dopamine) as indicated. The reaction was started by the addition of tissue extract (about 0.7 mg of protein). This was done within 5 min after the addition of $DMPH_4$ as the cofactor is unstable at neutral pH. After incubating at 37° for 40 min, the reaction was terminated by immersing the tubes in an ice bath followed by the addition of 2 ml of 5% (w/v) trichloroacetic acid. Blank activity was measured in an incubation mixture containing boiled tissue extract. The [^{14}C]-DOPA formed was separated from tyrosine by the method described by Nagatsu *et al.* [17] and the radioactivity measured in 10 ml of Bray's solution [18]. By this procedure, the recovery of DOPA was consistently 70 per cent. The results presented were corrected for recovery. Enzyme activity, expressed as pmol of [^{14}C]-DOPA formed/hr/mg protein, is given as the mean of duplicated assays.

Preparation of homogenates and synaptosomes for uptake and release studies. Striatal tissues from 2–4 rats were homogenized in 10 vol (w/v) of a modified Krebs phosphate buffer by 10 up-and-down strokes of a Teflon pestle rotating at about 800 rev./min in a smooth glass homogenizer (Thomas, U.S.A., type B, 0.1–0.2 mm clearance). The buffer contained 119 mM NaCl, 39 mM, KCl, 0.65 mM $MgSO_4$, 0.51 mM $CaCl_2$ and 19 mM sodium phosphate at pH 7.4.

Crude synaptosomal fractions (P_2) were obtained by differential centrifugation in a discontinuous sucrose gradient as described by Gray and Whittaker [19]. These preparations contained a large number of intact synaptosomes as revealed by the electron microscope. As shown by others [20], these synaptosomal preparations deteriorated only slowly during the first 5 hr after preparation, but after this, the ability to take up dopamine was rapidly lost. Hence, uptake studies were done within 5 hr after the preparation of synaptosomes.

Measurement of uptake of dopamine by homogenates and synaptosomes. Uptake of dopamine was studied by modification of the procedure of Holz and Coyle [20]. An aliquot of 0.1 ml of striatal homogenate (about 1 mg protein) or synaptosomes (about 80 μg protein) was added to 0.7 ml of the modified Krebs phosphate buffer supplemented with 10 mM glucose and 0.1 mg of ascorbic acid. Varying amounts of morphine and naloxone were added to reach a final volume of 0.9 ml. After preincubation at 37° for 1

min, 0.1 ml of [^{3}H]dopamine was added to reach final concentrations of 0.05 to 0.2 μM. At the end of the appropriate incubation periods, the contents were centrifuged at 10,000 *g* for 10 min at 4°. The pellet was washed twice with 1 ml modified Krebs phosphate buffer and then digested in 0.2 ml of 2.5 N NaOH by incubating at 65° for 30 min. Radioactivity in 0.1 ml aliquots of the digest was measured by scintillation counting in 5 ml of Bray's solution. The counting efficiency was 22 per cent determined by the use of internal standards. For studies of uptake by synaptosomes, the process was terminated by passing the incubation mixture through a Millipore filter (pore size 0.45 μm). The filter was washed twice with 1 ml of modified Krebs phosphate buffer containing 1 per cent bovine serum albumin. The processes of filtration and washing were completed in 10 sec. The filter was then dissolved in 5 ml Bray's scintillation solution and radioactivity measured with an efficiency of 27 per cent as determined by the use of internal standards.

The amount of non-specific adsorption of [^{3}H]dopamine by tissue homogenates or synaptosomes was routinely determined by using boiled tissue preparations (tissue blanks). The blank values were usually less than 10 per cent of the total uptake. The data presented have been corrected for blanks and counting efficiencies.

Measurement of release of dopamine by striatal homogenates and synaptosomes. To study spontaneous release, the striatal homogenate pellet, which was loaded with [^{3}H]dopamine as described above, was resuspended in 1 ml of modified Krebs phosphate buffer in the presence or absence of 100 μM morphine. When K^+-stimulated release was studied, the pellet was resuspended in 1 ml of modified Krebs phosphate buffer containing 120 mM KCl and 4 mM NaCl instead of the quantities described before. The pellet was dispersed by rapid mixing and incubated at 37° for the indicated period. The contents were then centrifuged at 10,000 *g* for 5 min at 4° and the radioactivities in the supernatant and the pellet were determined as before. For studies with synaptosomes, the Millipore filter containing synaptosomes loaded with [^{3}H]dopamine was placed in 2 ml of modified Krebs phosphate buffer in the presence or absence of 100 μM morphine. After 10 min of incubation at 37°, measurements were made of the radioactivity retained on the filter and that in the incubation medium. The extent of release of dopamine is expressed as:

$$\% \text{ dopamine released} = \frac{\text{radioactivity in incubation medium} \times 100}{\text{radioactivity in pellet (or filter)} + \text{radioactivity in incubation medium}}$$

Other analytical procedures. Results were examined for significance of differences by Student's '*t*' test. Protein contents were determined by the method of Lowry *et al.* [21] using bovine serum albumin as standard. Acetylated dopamine was identified by paper chromatography [22].

RESULTS

Effect of intracarotidly injected morphine on the levels of dopamine, homovanillic acid and cyclic AMP in the corpus striatum. As shown in Table 1, treatment with morphine caused profound changes in the striatal levels of dopamine, homovanillic acid and cyclic AMP. Five min after injection of the drug (5 mg/kg), these levels were raised to 180, 165 and 146 per cent of their respective saline controls.

Effect of morphine on TAT. In agreement with previous reports [9], striatal TAT was found to be a particulate enzyme which can be extracted with Triton X-100. The reaction mixture turned turbid upon the addition of KOH. Addition of ethanol helped to clarify the solution. Neither ethanol nor morphine (10–100 μM) interfered with the absorbance of *p*-hydroxyphenylpyruvate at 331 nm. However, in the presence of KOH, morphine absorbed significantly. Hence, morphine blanks were routinely included in studies on the effect of morphine on the activity of TAT. Under the assay conditions used, the enzyme activity was constant for up to 20 min; a reaction time of 15 min was used.

As shown in Table 2, morphine in the range of concentrations between 0.03 and 3 mM, had no effect on the activity of striatal TAT. It was also observed that neither the apparent K_m (5.0 mM) nor the V_{max} (8.3 μmol/hr/g tissue) for tyrosine was changed when morphine, at a final concentration of 10 or 100 μM, was present in the assays.

Effect of morphine on TH. The presence of $DMPH_4$ was essential for striatal TH activity. The omission of this cofactor from the assay mixture gave values

Table 1. Effect of intracarotidly injected morphine (5 mg/kg) on striatal dopamine, homovanillic acid and cyclic AMP levels

Treatment	Striatal metabolite contents		
	Dopamine (μg/g)	Homovanillic acid (ng/g)	Cyclic AMP (pmol/mg protein)
Saline control	6.0 ± 0.43 (9)	371.3 ± 13.7 (8)	33.29 ± 2.15 (8)
Morphine treated	10.8 ± 1.28 (6)†	613.6 ± 99.9 (5)*	48.76 ± 2.51 (8)‡

Values represent mean ± S.E.M. Figures in parenthesis indicate the number of experiments performed.
* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$, significantly different from control.

Table 2. Effect of morphine, added *in vitro*, on the activity of rat striatal TAT

Morphine concentration (mM)	0	0.03	0.1	0.3	3
TAT activity	4.12 ± 0.19	4.45 ± 0.20	4.17 ± 0.14	4.12 ± 0.14	4.29 ± 0.23

Each value is the mean of at least 3 experiments with S.E.M. indicated. TAT activity is expressed as μmol *p*-hydroxyphenylpyruvate formed/hr/g fresh weight of tissue.

Table 3. Effect of morphine, added *in vitro*, on the activity of rat striatal TH

Morphine concentration (μM)	TH Activity
Control (no morphine)	273 ± 10 (n = 7)
Morphine (1)	266 ± 17 (n = 5)
Morphine (10)	279 ± 15 (n = 5)
Morphine (100)	272 ± 12 (n = 5)

Each value is the mean of n experiments, with S.E.M. indicated. TH activity is expressed as pmol [^{14}C]-DOPA/hr/mg protein.

Table 4. Effect of morphine on the dopamine inhibition of rat striatal TH

Dopamine concentration (μM)	TH Activity	
	No morphine	With morphine (100 μM)
0	273 ± 10 (100)	272 ± 12 (100)
1	242 ± 5 (88)	238 ± 10 (87)
10	148 ± 7 (54)	154 ± 4 (56)
25	131 ± 8 (48)	123 ± 6 (45)
50	52 ± 3 (19)	55 ± 4 (20)
100	38 ± 1 (14)	41 ± 1 (15)

Each value represents the mean of at least 3 experiments with S.E.M. indicated. Figures in parenthesis are % enzyme activity as compared to maximal enzyme activity in the absence of added dopamine. TH activity is expressed as pmol [^{14}C]-DOPA/hr/mg protein.

Table 5. Effect of morphine on cyclic AMP stimulation of rat striatal TH

Cyclic AMP concentration (μM)	TH Activity	
	No morphine	With morphine (10 μM)
0	273 ± 10 (0)	272 ± 12 (0)
1	351 ± 12 (29)	398 ± 8 (46)*
10	353 ± 20 (29)	392 ± 19 (44)
100	385 ± 8 (41)	417 ± 11 (53)

Each value represents the mean of at least 3 experiments with S.E.M. indicated. Figures in parenthesis are % activation of enzyme activity as compared to the control enzyme activity in the absence of added cyclic AMP and morphine.

* $P < 0.05$, significantly different from the control activity with added cyclic AMP but no morphine. TH activity is expressed as pmol of [^{14}C]-DOPA/hr/mg protein.

comparable to the blanks. Under the assay conditions used, the enzyme activity was constant for up to 40 min. The blank values were independent of incubation time. This indicated that there was little or no non-specific formation of [^{14}C]-DOPA under these conditions. None of the test substances, including morphine (100 μM), dopamine (100 μM), cyclic AMP (100 μM) ATP (0.5 mM) and Mg^{2+} (20 mM), interfered with the adsorption of DOPA on or elution from alumina.

The activity of striatal TH was generally not affected by morphine. The activity of the enzyme from tissues of animals which had received morphine (5 mg/kg, intracarotid, 5 min) was 266 ± 16 pmol DOPA/hr/mg protein which was the same (260 ± 14) as that from control animals. Morphine, added *in vitro* at final concentrations of 1–100 μM, did not alter the activity of TH (Table 3). The results also revealed that surgical operations, including the application of pentobarbital anaesthesia, did not affect the activity of striatal TH significantly.

Striatal TH was strongly inhibited by dopamine. For example, in the presence of 100 μM dopamine, the enzyme activity was only 14 per cent of the control (Table 4). This inhibitory effect was shown not to be altered in the presence of morphine (100 μM), studied over a range of dopamine concentrations (1–100 μM).

Cyclic AMP, at final concentrations of 1–100 μM, stimulated the striatal TH activity (Table 5). This effect could only be demonstrated if Mg^{2+} and ATP were added. In the presence of morphine (100 μM), the stimulatory effect was somewhat enhanced. However, this enhancement of cyclic AMP stimulation by morphine was only significant at the lower cyclic AMP concentrations. At higher concentrations, the enhancement was statistically insignificant.

Effect of morphine on the uptake of [^{3}H]*dopamine by striatal homogenates and synaptosomes.* At 37°, striatal homogenate took up [^{3}H]dopamine at a rate of 1.87 pmol/min/mg protein. This rate was constant for up to 10 min at incubation. At 4°, the rate was comparable to the tissue blank. The dopamine taken up was found to be associated with the synaptosomal fraction upon analysis by differential centrifugation of the [^{3}H] dopamine loaded homogenate in a discontinuous sucrose gradient [19]. Morphine, when present at concentrations of 50 and 100 μM, was found to inhibit the rate of uptake of [^{3}H]dopamine by 14 per cent and 33 per cent respectively. However, as shown in Table 6, the inhibition was not reversed by the addition of 100 μM naloxone.

The uptake of [^{3}H]dopamine was more active in

Table 6. Effects of morphine and naloxone on the uptake of [^{3}H]dopamine by rat striatal homogenates

Drug concentrations (μM)	Rate of [^{3}H]dopamine uptake (pmol/min/mg protein)
Morphine (0) – control	1.87 ± 0.05
Morphine (50)	1.60 ± 0.05*
Morphine (100)	1.26 ± 0.12†
Morphine (100) + Naloxone (100)	1.32 ± 0.09†

The concentration of [^{3}H]dopamine used was 0.1 μM. The values are the mean ± S.E.M. of 2–3 determinations performed in duplicate.

* ($P < 0.05$) and † ($P < 0.01$), significantly different from the control.

synaptosomal fractions than in homogenates. The rate was found to be proportional to the amount of synaptosomes present in the range of 40–160 μg of protein. The rate measured in the presence of 80 μg protein, was constant for 2 min and then became non-linear at longer incubation times. The uptake by synaptosomes was at least twice as fast at 37° than at 20°. At 4°, the rate was the same as the tissue blank. Chromatographic analysis indicated that all of the radioactivity was accounted for in the band corresponding to acetylated dopamine. This suggested that the dopamine taken up by the synaptosomes was not changed. It was found that uptake of dopamine by synaptosomes was also inhibited by morphine (Table 7). In the presence of 100 μM morphine, the rate was diminished by 17 per cent. The inhibition was less significant at lower morphine concentrations. The Lineweaver–Burk plot shown in Fig. 1 demonstrated an increase in the apparent K_m from 0.14 μM to 0.17 μM in the presence of 100 μM morphine while the V_{max}, 77 pmol/min/mg protein, was not changed. A study of the inhibitory effect of different concentrations of morphine on the uptake at two concentrations of dopamine produced an apparent K_i value of 0.41 mM (Fig. 2).

To determine whether morphine caused osmotic lysis of synaptosomes and hence an apparent inhibition of dopamine uptake, synaptosomes were incubated in normal medium containing an additional 100 mM sucrose [20]. Under these conditions, the

Table 7. Effects of morphine and naloxone on dopamine uptake by striatal synaptosomes

Drug concentration (μM)	Rate of [^{3}H]dopamine uptake pmol/min/mg protein
Morphine (0)-control	32.0 ± 0.6
Morphine (1)	30.4 ± 0.7
Morphine (10)	29.9 ± 0.9
Morphine (100)	26.5 ± 0.5*
Naloxone (100)	31.3 ± 0.4
Morphine (100) + Naloxone (100)	25.9 ± 0.6*

The concentration of [^{3}H]dopamine used was 0.1 μM. The values are the mean ± S.E.M. of 3–5 determinations performed in duplicate.

* ($P < 0.001$), significantly different from the control.

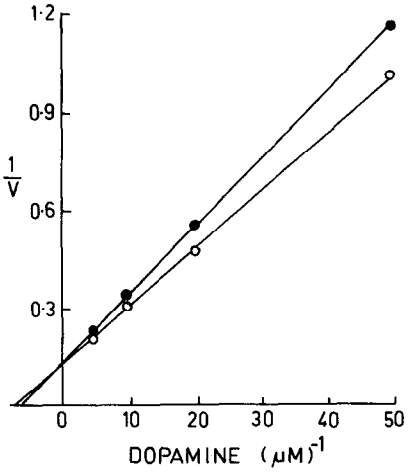

Fig. 1. Double reciprocal plot of initial rate of uptake of dopamine (v = pmol[^{3}H]dopamine/min/100 μg protein) by striatal synaptosomes in the presence (●) or absence (○) of 100 μM morphine as a function of dopamine concentration. Each point represents the mean of 3 separate experiments.

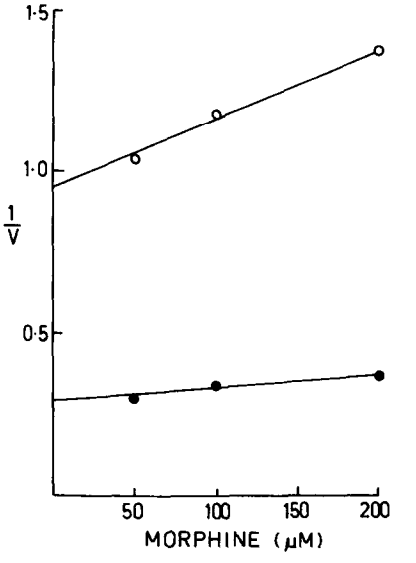

Fig. 2. Dixon's plot of the reciprocals of initial rates of uptake of dopamine (v = pmol[^{3}H]dopamine/min/100 μg protein) by striatal synaptosomes as a function of the morphine concentration. The dopamine concentration was fixed at 0.02 (○) and 0.1 μM (●). Each point represents the mean of duplicated determinations.

uptake was reduced by 48 per cent but the extent of inhibition by morphine was the same as that measured under normal conditions (Table 8). The observed inhibition could also be caused by an immediate release of recently taken up dopamine. Since exocytotic release is Ca^{2+}-dependent, the effects of morphine on the rates of uptake under normal conditions and in an environment depleted of Ca^{2+} were compared. These were again found to be similar (Table 8).

Effect of morphine on spontaneous and K^+*-stimulated release of dopamine by striatal homogenate and*

Table 8. Lack of effect of sucrose and Ca^{2+} on morphine inhibition of [^{3}H]dopamine uptake by striatal homogenates

Conditions	No sucrose normal Ca^{2+}		100 mM sucrose		No sucrose No Ca^{2+} + 2 mM EGTA	
	uptake	relative uptake	uptake	relative uptake	uptake	relative uptake
Control	32 ± 0.6	1	16.6 ± 0.3	1	33.1 ± 0.7	1
Morphine (100 μM)	26.5 ± 0.4	0.83	14.1 ± 0.4	0.85	26.4 ± 0.3	0.80

Concentration of [^{3}H]dopamine used was 0.1 μM. Each value represents the mean of 3–5 determinations performed in duplicate. The rates of uptake were expressed as pmol [^{3}H]dopamine/min/mg protein.

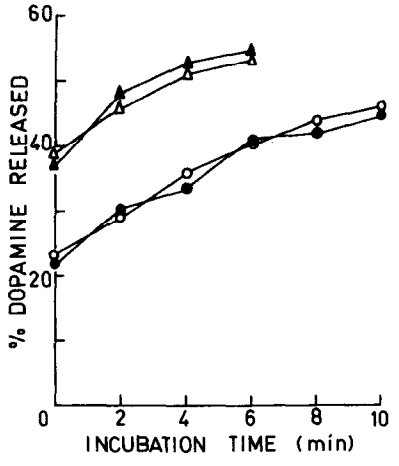

Fig. 3. Effect of morphine (100 μM) on the spontaneous (○) and K^+ stimulated (△) release of dopamine by striatal homogenate. The dopamine concentration was 0.05 μM. The values are means of 3–5 experiments. The filled symbols represent release in the presence of morphine.

synaptosomes. Morphine, at 10–100 μM, has no effect on both the spontaneous and K^+-stimulated release of dopamine by striatal homogenates. Figure 3 illustrates the comparable rates of release measured in the presence or absence of 100 μM morphine. Similarly, using striatal synaptosomal preparations, 52 ± 4% of [^{3}H]dopamine was released under normal conditions as compared with a value of 54 ± 3% (Mean ± S.E.M., n = 3) in the presence of 100 μM morphine.

DISCUSSION

There are strong reasons to believe that brain dopaminergic mechanisms are involved in the actions of narcotic drugs. It is generally agreed that acute administration of morphine increases the turnover of dopamine [4]. Results which showed an increased incorporation of [^{14}C]tyrosine into [^{14}C]dopamine [23, 24] or increased rate of depletion of dopamine [25] in brain tissues after morphine treatment are consistent with this hypothesis. Results of studies on changes in the content of dopamine are less demonstrative. Thus, morphine is known to decrease the dopamine level in the mouse brain [26]. The drug may increase [24, 27] or have no effect on [25, 28] the dopamine level in the rat striatum depending on how soon the measurements were made after drug administration. There is a better agreement that morphine causes an increase in the level of homovanillic acid [25, 29]. This compound is the major metabolite of dopamine in rat striatal tissues. It largely represents dopamine metabolized after release from nerve endings and hence can be used as an index of dopaminergic activity.

In the present study, the levels of striatal dopamine and homovanillic acid were measured 5 min after the rat received an intracarotid injection of morphine. Under the same conditions, the drug was shown to suppress the spontaneous unitary discharges of caudate neurons [1–3]. The levels of both of these compounds were found to be increased drastically. These neurochemical changes are consistent with the hypothesis that the suppressive effect of morphine was probably due to an increase in dopaminergic activities in the nigrostriatal pathway [3].

In the rat striatum, the postsynaptic dopamine receptor may be functionally coupled to a dopamine-sensitive adenylate cyclase system [30]. The effect of morphine on striatal cyclic AMP content was studied because enhancement of dopaminergic activity should be accompanied by an elevation in the cyclic AMP level in the target organ. This increase was indeed observed together with the rises in the levels of dopamine and homovanillic acid. Other workers have also found that morphine causes a transient rise in cyclic AMP content in the rat brain although the drug was administered by other routes and at higher dosages [31, 32]. It can be argued that the increase is due to a direct stimulation of the basal or dopamine-stimulated adenyl cyclase activity. However, the effect of morphine on both of these activities as well as the cyclic AMP phosphodiesterase activity is uncertain [33]. We observed that these activities were not affected by acute morphinization or if the drug was added *in vitro* (unpublished data). It is noted that the normal striatal cyclic AMP levels observed here are several-fold higher than the values of 5–12 pmol/mg reported by others [32, 34]. The discrepancy may be explained by the fact that cyclic AMP may accumulate by 8-fold within 90 sec after decapitation [35].

One of the ways leading to an increase in dopamine synthesis is by an increased availability of tyrosine. A tyrosine pool of 0.1 mM has been measured in the brain [36, 37] and the K_m of striatal TAT for tyrosine is some 500-fold higher than that of TH [17, 38]. These observations suggest that if tyrosine is evenly

distributed, striatal TH is normally saturated with the substrate and any alteration in brain tyrosine level will not greatly influence dopamine synthesis. However, both DOPA and dopamine were found to accumulate appreciably in the rat striatum following an intracisternal injection of tyrosine [23]. This indicates that the tyrosine concentration may not normally be saturating for striatal TH. Transamination may, therefore compete with the catecholamine biosynthetic pathway for the pool of tyrosine [9, 10]. If morphine decreases the transamination activity, more tyrosine would be available for dopamine synthesis. Such a case was not found and neither the K_m nor the V_{max} of TAT for tyrosine was changed in the presence of morphine. Acute morphinization has been shown to increase the specific radioactivity of brain [^{14}C]tyrosine [39]. Since the brain concentrates L-tyrosine from the circulation by a stereospecific transport system [36], a direct action of morphine on this process to increase tyrosine availability in the brain cannot be ruled out. It is also possible that morphine may increase the retention of tyrosine in the brain [40].

Morphine, added *in vitro*, or by acute treatment, did not affect the activity of TH [41, 42]. These observations are confirmed in the present study. The activity, measured 5 min after intracarotid injection of the drug, was also not changed. Thus, increases in the levels of dopamine and homovanillic acid in the striatum after such treatment with the drug cannot be explained by a direct stimulation of TH. It is also unlikely that the increases were due to a change, by morphine, in the degree of feedback inhibition of TH by dopamine (Table 4, [42]). The activity of the enzyme has been found to be stimulated by cyclic AMP [43]. This effect was believed to be mediated by a phosphorylation reaction through the action of a protein kinase [44]. This effect was again demonstrated in this study although a clear cut dosage effect was not apparent. This could be explained by the presence of cyclic nucleotide phosphodiesterase. Nevertheless, morphine was found to potentiate, slightly but significantly, the stimulatory effect of cyclic AMP.

Studies with striatal slices revealed an inhibitory action of morphine on K^+-stimulated release of dopamine [45–47]. Acute morphinization has also been reported to lower the spontaneous release of primary amines from caudal slices [48]. In the present study, neither the spontaneous nor K^+-stimulated release of dopamine from striatal homogenates or synaptosomal fractions was affected by morphine at concentrations as high as 100 μM. The reason for this discrepancy is not clear. One possible explanation is loss of neuronal integrity upon homogenization. On the other hand, morphine was observed to inhibit the uptake of dopamine by these preparations. This effect was demonstrated in mouse brain cortex slices [49] whereas the uptake of dopamine into rat striatal nerve endings has been shown to be inhibited by morphine only at high concentrations of dopamine when diffusion is the important component of the transport system [50]. The observed inhibitory effect was not an artifact due to morphine-induced osmotic lysis of the synaptosomes or Ca^{2+}-dependent exocytic release of recently taken up dopamine (Table 8).

The dopamine uptake system at the presynaptic terminals may contribute to two important regulatory mechanisms, viz. the inactivation of the transmitter at the synapse and the inhibition of TH, the rate-limiting enzyme for catecholamine synthesis. Dopamine was shown to be incorporated into rat striatal synaptosomes very efficiently (K_m = 0.14 μM). Once taken up, dopamine may be stored in synaptic vesicles or catabolized by relevant enzymes. At least in the pig brain, the K_m of MAO for dopamine was about 100 μM [51]. The K_m for dopamine uptake by rat cerebrum synaptic vesicles has been reported to be 1.6 μM [52]. It is therefore very likely that most of the dopamine taken up by synaptosomes remained in the cytosol. If this is true, then the impairment of dopamine uptake by morphine would result, on the one hand, in an enhancement and prolongation of the effect of dopamine on the post-synaptic neurons, and on the other hand, a relative decrease in presynaptic cytosol dopamine level. The former effect would lead to an increased cyclic AMP level due to a dopamine-stimulated adenylate cyclase [53] which would then result in an enhancement of TH activity in the post-synaptic neuron. This mechanism is consistent with the finding that an increased impulse flow in the nigrostriatal pathway activated striatal TH [54]. The latter effect would result in a decrease in feedback inhibition of the activity of TH in the presynaptic neuron leading to increased synthesis of dopamine. These proposed mechanisms would explain increased dopaminergic activities and dopamine, homovanillic acid and cyclic AMP levels in the striatum upon acute morphinization.

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