THE EFFECT OF MORPHINE ON RAT BRAIN CATECHOLAMINES: TURNOVER *IN VIVO* AND UPTAKE IN ISOLATED SYNAPTOSOMES*

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THE ACUTE administration of morphine and other narcotic analgesic drugs to animals produces transient changes in the levels of biogenic amines in the central nervous system (WAY and SHEN, 1971). Among the most striking acute responses are the depletion of dopamine from the striatum (GUNNE *et al.*, 1969) and norepinephrine from the hypothalamus (VOGT, 1954; REIS *et al.*, 1969). As measured by the accumulation of ^{14}C-dopamine and -norepinephrine from ^{14}C-tyrosine *in vivo*, the biosynthesis of dopamine is transiently increased after a single injection of morphine, especially in the hypothalamus and striatum (CLOUET and RATNER, 1970). A more pronounced increase in the rates of dopamine biosynthesis was found in the same brain regions of morphine-tolerant rats.

The present report is the results of the exploration of the relationship between brain biogenic amines and opiates in three aspects: (1) biosynthesis of catecholamines; (2) tyrosine hydroxylase levels; and (3) synaptosomal uptake of catecholamines.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats were injected subcutaneously with morphine sulphate in doses of 5, 20 or 60 mg/kg, which produces analgesic and hypothermic responses in this strain for 63, 138 and 192 min, respectively. After sacrifice, the brains were removed and dissected into six regions (GLOWINSKI and IVERSEN, 1966). Chronic treatment with morphine was by daily injection of 60 mg/kg/day or by pellet implantation.

Catecholamine biosynthesis

^{14}C-tyrosine was administered intracisternally under light ether anaesthesia for a 10 min period. Norepinephrine (NE) and dopamine (DA) levels were measured by the method of ANTON and SAYRE (1962) after separation on Dowex-50 (NYBACK and SEDVALL, 1968), and the fractions examined for radioactivity by scintillation spectrometry. Protein was measured by the Folin method of LOWRY *et al.* (1951).

Tyrosine hydroxylase

The sum of the accumulations of ^{14}C radioactivity in dopa, DA and NE in a 10 min period *in vivo* was taken as an estimation of tyrosine hydroxylase *activity* during the period. The enzyme *levels* were assayed in brain areas in independent experiments using a 40 per cent saturated ammonium sulphate precipitate, as described by MUSACCHIO *et al.* (1969).

Catecholamine uptake

Pooled samples of striatum (for DA) and hypothalamus (for NE) were homogenised and centrifugally fractionated to sediment either a crude mitochondrial fraction containing the synaptosomes or a purer synaptosomal fraction isolated by centrifuging the crude mitochondrial fraction through a sucrose density gradient (HAGA, 1971). The synaptosomes were incubated in Krebs-Henseleit medium with 2×10^{-6} M amine and the MAO inhibitor, nialamide, for 5 min at 37° in the presence or absence of opiates. The reaction was stopped by separating the particles and medium by Millipore® filtration, and each fraction was examined for radioactivity.

RESULTS AND DISCUSSION

The levels of NE were increased in the hypothalamus after a single injection of morphine (Table 1). DA levels were too low to measure, except in the striatum, in which the levels were significantly lower at 30 min, and above control at 60 min. The rates of biosynthesis of norepinephrine were relatively unaffected by morphine. Dopamine biosynthesis was increased in most areas after a single injection of 60 mg/kg of morphine, and in all areas in tolerant animals (Table 1). After 5 or 20 mg/kg, the responses were in the same direction but less, except for dopamine biosynthesis which was even faster after 20 mg/kg than after 60 mg/kg.

The results suggest that the responses of the dopaminergic system are predominant after morphine administration.

TABLE 1. CHANGES EFFECTED BY MORPHINE ADMINISTRATION IN CATECHOLAMINE LEVELS AND RATES OF BIOSYNTHESIS

	Cerebellum	Medulla	Hypothalmus	Striatum	Midbrain	Cortex
NE Levels (μg/g)	163 ± 2	587 ± 37	1144 ± 53	221 ± 25	459 ± 25	210 ± 12
Acute morphine	*ns*	*ns*	1943 ± 221	*ns*	*ns*	297 ± 26
Chronic morphine pellets	*ns*	429 ± 34	1599 ± 192	*ns*	344 ± 41	250 ± 34
DA Levels (μg/g)				2067 ± 44		
Acute morphine						
30 min				1005 ± 112		
60 min				2427 ± 189		
Chronic morphine pellets				2439 ± 90		
NE Synthesis (nmoles/hr/mg protein)	0·8 ± 0·0	5·1 ± 0·2	21·0 ± 2·0	174 ± 24	23 ± 1·0	0·23 ± 0·0
DA Synthesis (nmoles/hr/mg)	8·9 ± 0·7	13·7 ± 0·6	72·4 ± 8·0	24·1 ± 3·0	10·3 ± 0·5	26·2 ± 3·0
Acute morphine						
30 min	*ns*	*ns*	90·9 ± 1·0	*ns*	12·2 ± 0·2	*ns*
60 min	*ns*	*ns*	119·7 ± 23	33·8 ± 0·3	*ns*	*ns*
Chronic morphine pellets	13·8 ± 2·0	23·1 ± 0·6	118·9 ± 5·0	38·5 ± 3·0	18·1 ± 1·0	49·3 ± 5·0

The levels and turnover values are averages of at least three experiments.
Only values different from control values at $P < 0.01$ are listed.
ns = not significant.

TABLE 2. THE EFFECT OF MORPHINE ADMINISTRATION ON TYROSINE HYDROXYLASE IN BRAIN
Tryosine hydroxylase (nmoles/hr/g wet weight)

	Cerebellum	Medulla	Hypothalamus	Striatum	Midbrain	Cortex
Controls						
in vivo	0·43	0·99	4·14	1·34	1·40	1·77
in vitro	2·12	4·71	9·98	12·48	12·29	3·82
Acute						
in vivo	0·41	0·89	4·61	2·57*	1·53	1·50
in vitro	1·86	5·27	9·85	10·39	12·81	4·07
Chronic-injected						
in vivo	0·59*	1·39*	5·61*	3·79*	1·84*	2·20*
in vitro	1·19	5·27	10·37	12·97	10·62	3·87
Chronic-pellets						
in vivo	0·46	0·83	3·32*	2·14*	1·20	2·06
in vitro	1·66	5·48	13·17*	17·40*	10·73	3·89

The starred values are different from controls at $P < 0{\cdot}01$.

The *in vivo* accumulation of ^{14}C in dopa, DA and NE, and the *in vitro* assay for tyrosine hydroxylase are described in the methods section. The acute treated rats were injected with 60 mg/kg morphine and killed 1 hr later. The chronically injected animals were injected with 60 mg/kg morphine each day for four (*in vivo*) or five (*in vitro*) days and sacrificed 2 hr after the last dose.

Because the rates of synthesis of the individual catecholamines varied widely during morphine treatment, the sums of ^{14}C-catecholamines synthesised from tyrosine also varied widely, with significant differences in each area at some point after morphine injection. The most pronounced increase in tyrosine hydroxylase activity was found in the brains of rats killed 2 hr after the fourth daily injection of morphine (Table 2). The actual *levels* of tyrosine hydroxylase, however, were remarkably constant during morphine treatment, with significant increases in isolated enzyme activity only in the striatum and hypothalamus of long-time morphine-treated rats (Table 2). These increases may be taken as an indication that tyrosine hydroxylase was induced during chronic morphine treatment. It is possible that induction at lower levels occurred at earlier times (or in other tissue areas) in amounts too low to be detectable.

The nature of the opiate-catecholamine interaction was not defined by the results of the previous studies. In order to explore the possibility that narcotic analgesics have a direct action on the transport of catecholamines across the neuronal membranes, the effect of morphine on the uptake and release of the amines in isolated synaptosomes was examined. The rates of uptake of ^{14}C-NE into hypothalamic synaptosomes and ^{14}C-DA into striatal synaptosomes were inhibited in the presence of morphine-HCl, but only at concentrations of 10^{-4} M or higher. When this inhibition was examined in relation to DA concentration, it was found that only the low affinity uptake, representing diffusion, was inhibited by morphine. These results suggest that one effect of morphine *in vivo* is an interference with dopamine re-uptake following after an excessive release of the amine triggered by a prior event in the central nervous system. The rates at which the biogenic amines were released after preloading with labelled amines *in vitro* was not affected by morphine.

SUMMARY

(1) Acute morphine administration produced an initial depletion of DA in the striatum and an increased rate of DA biosynthesis in the same area. In tolerant animals, the biosynthesis of DA was increased in all areas of rat brain.

(2) While the *activity* of tyrosine hydroxylase varied widely during morphine treatment, the *levels* were increased in some brain areas only after long-time morphine-treatment.

(3) The uptake of NE and DA into isolated synaptosomes was inhibited by morphine at concentrations of 10^{-4} M or higher. A kinetic analysis of DA uptake in the presence of morphine suggested that DA re-uptake by diffusion was the mode of transport inhibited by the opiate.

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