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PHYSICAL DEPENDENCE ON MORPHINE FAILS TO INCREASE SEROTONIN TURNOVER RATE IN RAT BRAIN*

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Abstract—Rats were made dependent on morphine by pellet implantation. Injections of nalorphine (50 mg/kg, i.p.) elicited a withdrawal syndrome characterized by hypothermia, salivation, lacrimation, urge to escape, wet dog shakes and squeaking. Pretreatment with *p*-chlorophenylalanine (300 mg/kg, i.p./day for 5 days) did not lessen the severity of the withdrawal precipitated by nalorphine. Isotopic and nonisotopic measurement of brain norepinephrine and serotonin turnover rates shows that morphine dependence fails to change these rates.

BY MEASURING the rate of accumulation of brain serotonin after inhibition of monoamine oxidase (MAO), Way *et al.*[1] concluded that mice made physically dependent to morphine have an augmented turnover rate of brain serotonin. Moreover, when serotonin synthesis is inhibited by injecting *p*-chlorophenylalanine in mice dependent on morphine, the severity of the withdrawal syndrome precipitated by naloxone injections is reduced.[1] Other authors[2–4] reported that treatment of rats and mice with protein synthesis inhibitors blocks the development of tolerance to morphine. Loh *et al.*[5] have obtained similar effects also on the development of physical dependence and have inferred that the protein essential to develop both tolerance and dependence to morphine may be a protein functioning in the control of brain serotonin synthesis. We have extended the study of brain serotonin turnover rate to rats physically dependent to morphine. We now report that brain serotonin turnover rate is unchanged in rats physically dependent on morphine. Moreover, in rats implanted with morphine pellets, the inhibition of brain serotonin synthesis with *p*-chlorophenylalanine fails to attenuate the severity of the withdrawal syndrome precipitated by injections of *N*-allynormorphine (nalorphine). We conclude that the increase of brain serotonin synthesis occurring in mice physically dependent on morphine might be a phenomenon restricted to certain animal species, as we have found that in rats implanted with morphine pellets the turnover rate of brain serotonin (5-HT) is equal to that of sham-operated rats.

METHODS

Sprague–Dawley rats, male, 180–200 g, were employed. We obtained physical dependence to morphine by modifying the Huidobro and Maggiolo morphine

* A preliminary report of these experiments was presented at the Fall Meeting of the American Physiological Society, Davis, Calif. (1969).

implantation technique[6] as described by Way *et al.*[1] Our procedure involves local anesthesia and subcutaneous implantation in the lower side of the abdominal wall of a specially formulated pellet of morphine base (75 mg). This first implantation is followed by a second implantation 70 hr later in the contralateral side. The physical dependence to morphine is verified about 120 hr after the first morphine implantation by injecting 50 mg/kg, i.p. of nalorphine. Control rats were sham-implanted and both groups of rats received 10,000 i.u. of penicillin twice daily throughout the experiment. Evaluation of the withdrawal syndrome is made by placing the rats in single cages, measuring the rectal temperature, and noting the behavioral responses before and at various times after nalorphine. In rats, the withdrawal syndrome precipitated by nalorphine is characterized by diarrhea, repeated attempts to escape from the cage, lacrimation, salivation, squeaking when handled, hypothermia and, most characteristically, by the wet dog shakes described previously by other authors.[7,8] The severity of this syndrome was rated, giving to the appearance of each symptom in each rat a score of one. Brain 5-HT turnover rate was estimated by comparing the amine concentration in brains removed from sham-operated and morphine-implanted rats at various times after the intraperitoneal injection of 75 mg/kg, i.p., of pargyline. In addition, the turnover rate of brain 5-HT and norepinephrine (NE) was measured by an isotopic technique. In brief, this technique requires the intravenous injections of 500 μc/kg of (^{3}H)-L-tryptophan generally labeled (5 c/m-mole) and 100 μc/kg of [^{14}C]-L-tyrosine generally labeled (350 mc/m-mole). Rats are sacrificed 5, 10 and 15 min after labeling and the specific activity of plasma tyrosine, brain tryptophan, 5-HT and NE in brain is determined. Estimations of fractional rate constants of monoamines (k_m) efflux was obtained from

$$k_m \approx \frac{\dfrac{M_{t_{15}} - M_{t_5}}{t_{15} - t_5}}{\dfrac{(AA - M)t_5 + (AA - M)t_{15}}{2}} \quad . \tag{1}$$

The derivation of this difference equation from

$$\frac{dM}{dt} = k_m (AA - M) \tag{2}$$

was reported previously.[9] In equations (1) and (2), M = brain monoamines specific activity; AA = plasma or brain amino acid specific activity, t_n = time of specific activity measurements where n = minutes after the injection of the label.

In another group of experiments, we tested whether the rate of 5-HT synthesis was related to the severity of the syndrome precipitated by nalorphine. Rats chronically treated with morphine were divided into two groups: one received 300 mg/kg, i.p., of *p*-chlorophenylalanine (PCPA) every day for 5 days beginning the day preceding the morphine implantation; the other group received a corresponding volume of vehicle. The severity of the withdrawal syndrome was verified by comparing the severity of the syndrome in the two groups of rats.

RESULTS

Withdrawal syndrome precipitated by nalorphine. The data listed in Table 1 rate the responses elicited by nalorphine injected to sham-operated and morphine-implanted

TABLE 1. EFFECTS OF *N*-ALLYLNORMORPHINE (NAM) IN SHAM-OPERATED AND MORPHINE-IMPLANTED RATS*

Morphine-implanted	NAM	Time after NAM (min)	No. of rats showing					
			Salivation	Lacrimation	Urge to escape	Wet dog shakes	Diarrhea	Squeaking
No	Yes (5)	15	0	0	0	0	0	0
No	Yes (5)	180	0	0	0	0	0	0
Yes	Yes (5)	15	5	4	5	5	5	4
Yes	Yes (5)	180	0	0	0	3	0	3

* One 75 mg morphine pellet was implanted on days 1 and 3, and on day 5 these rats were injected with 50 mg/kg (i.p.) of NAM. In parentheses, number of sham-operated and morphine-implanted rats injected with NAM.

rats. These data show that nalorphine does not elicit salivation, lacrimation, wet dog shakes, diarrhea and squeaking in sham-operated rats, whereas in morphine-treated rats this symptomatology may persist for about 3 hr. Besides the wet dog shakes described by other authors,[7,8] as characteristic of the withdrawal syndrome precipitated by nalorphine, the rats evinced an urge to escape. Soon after injections of nalorphine, the rats appear increasingly restless. With time they continuously attempt to get out of the cages by pushing on the lids. Morphine-implanted rats receiving nalorphine squeak when they are handled. Salivation, lacrimation and diarrhea represent the neurovegetative component of the withdrawal syndrome precipitated by nalorphine.

Table 2 lists the rectal temperature measurements taken in sham-operated and morphine-implanted rats before and after nalorphine injections. In confirmation of

TABLE 2. BODY TEMPERATURE OF SHAM-OPERATED AND MORPHINE-IMPLANTED* RATS AT VARIOUS TIMES BEFORE AND AFTER *N*-ALLYLNORMORPHINE (NAM) INJECTIONS

Time before (−) or after (+) NAM (min)	Mean rectal temperature (°F ± S.E.)	
	Morphine-implanted (5)	Sham (5)
− 120	101 ± 0·4	99·1 ± 0·3†
− 5	101·2 ± 0·2	99·1 ± 0·2†
+ 30	96·7 ± 1‡	100·7 ± 1·1
+ 180	98·7 ± 0·8‡	99 ± 0·3

* See Methods and Table 1 for morphine implantation and precipitation of morphine withdrawal with NAM. In parentheses, number of sham-operated and morphine-implanted rats injected with NAM.

† $P > 0.05$ when compared with temperature of animals given morphine.

‡ $P < 0.05$ when compared with temperature before NAM injections.

previous reports[7] with morphine-dependent rats, the rectal temperature of our morphine-implanted rats was higher than that of sham-operated animals. When both groups of rats were injected with nalorphine, only the morphine-implanted rats exhibited a significant hypothermia. The rectal temperature of the morphine-dependent rats, originally higher than that of sham-operated rats, reaches abnormally low values within 30 min after the nalorphine injection. This hypothermia, like many other signs of the withdrawal syndrome precipitated by nalorphine, lasted about 3 hr.

The data reported in Table 3 show that *p*-chlorophenylalanine, administered in doses that lower brain 5-HT concentrations[10] and reduce its turnover by 80 per cent or more,[11] does not change the severity of the withdrawal syndrome precipitated by nalorphine injection given to morphine-implanted rats.

TABLE 3. EFFECT OF *p*-CHLOROPHENYLALANINE (PCPA) ON SYMPTOMS ELICITED BY *N*-ALLYLNORMORPHINE (NAM) IN RATS MADE TOLERANT TO MORPHINE

Treatment†	No. of rats showing					
	Salivation	Lacrimation	Urge to escape	Wet dog syndrome	Diarrhea	Squeaking
Sham (3)	0	0	0	0	0	0
PCPA (3)	0	0	0	0	0	0
Morphine, PCPA (3)	2	2	3	3	1	3
Morphine (3)	2	2	2	3	1	3

* See Methods and Table 1 for morphine implantation and precipation of withdrawal by NAM. The rats received one injection of PCPA (300 mg/kg) i.p. every day for 5 days. The withdrawal from morphine was elicited by administration of NAM (50 mg/kg, i.p.).

† Number of animals in parentheses. Responses recorded 30 min after NAM.

TABLE 4. BRAIN 5-HT CONCENTRATIONS IN SHAM-OPERATED AND MORPHINE-IMPLANTED RATS RECEIVING PARGYLINE*

Treatment	Brain 5-HT (mμmoles/g ± S.E.) Minutes after pargyline			Turnover rate (mμmoles/g/hr)
	0	15	60	
Sham-operated	2·5 ± 0·11 (4)	3·1 ± 0·09 (4)	4·3 ± 0·17 (4)	1·8
Morphine-implanted	2·3 ± 0·07 (4)	2·9 ± 0·13 (4)	4·0 ± 0·19 (4)	1·7

* Pargyline (75 mg/kg, i.p.) was injected in sham-operated and morphine-implanted rats on the fifth day after surgery (see Methods and Table 1 for schedules of morphine implantation). Number of animals in parentheses.

Brain 5-HT turnover rate in rats physically dependent on morphine. We have injected rats made physically dependent on morphine with 75 mg/kg, i.p., of pargyline and measured the brain 5-HT concentration at various times after the injection of the MAO inhibitor. As reported by Tozer *et al.*,[12] the accumulation of brain 5-HT with

TABLE 5. TURNOVER RATE OF BRAIN 5-HT IN RATS IMPLANTED WITH MORPHINE PELLETS*

Treatment	Time after [^{3}H]-tryptophan injection (min)	Brain tryptophan		Brain 5-HT			
		(mμmoles/ml ± S.E.)	(dis./min/mμmole ± S.E.)	(mμmoles/g ± S.E.)	(dis./min/mμmole)	$k_{5\text{-HT}}$ (hr^{-1})	Turnover rate (mμmoles/g/hr)
Sham-operated (4)	5	104 ± 13	18,850 ± 1550	2·02 ± 0·17	2050 ± 530		
Sham-operated (4)	10	120 ± 9	12,100 ± 1300	2·05 ± 0·17	2700 ± 480	1·09	2·25
Sham-operated (4)	15	113 ± 11	8650 ± 1000	2·15 ± 0·37	4000 ± 740		
Morphine (5)	5	100 ± 9	17,300 ± 1200	2·20 ± 0·15	2100 ± 470		
Morphine (5)	10	117 ± 6	11,500 ± 800	2·22 ± 0·17	2800 ± 200	0·81	1·80
Morphine (5)	15	115 ± 4	8400 ± 1300	2·30 ± 0·20	3500 ± 700		

* See Methods and Table 1 for schedule of morphine implantation and labeling with amino acids precursors. Experiments were performed 120 hr after the implantation of the first morphine pellet.

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TABLE 6. TURNOVER RATE OF BRAIN NOREPINEPHRINE IN RATS IMPLANTED WITH MORPHINE PELLETS*

Treatment	Time after [^{14}C]-tyrosine injection (min)	Plasma tyrosine		Brain norepinephrine			
		(mμmoles/ml ± S.E.)	(dis./min/mμmole ± S.E.)	(mμmoles/g)	(dis./min/mμmole ± S.E.)	k_{NE} (hr^{-1})	Turnover rate (mμmoles/g/hr)
Sham-operated (4)	5	58·5 ± 8·2	2002 ± 166	2·3 ± 0·2	34 ± 5·4		
Sham-operated (4)	10	57·8 ± 7·8	1215 ± 151	2·5 ± 0·1	46 ± 4·4	0·32	0·75
Sham-operated (4)	15	63 ± 2·8	873 ± 154	2·3 ± 0·1	106 ± 17		
Morphine (5)	5	106 ± 4·5†	1217 ± 29†	2·2 ± 0·1	39 ± 4·8		
Morphine (5)	10	70 ± 4·1†	1141 ± 46	2·2 ± 0·1	52 ± 4·3	0·36	0·79
Morphine (4)	15	70 ± 3·5†	609 ± 69	2·3 ± 0·2	90 ± 6·3		

* See Methods and Table 1 for schedule of morphine implantation and labeling with amino acid precursors. Experiments were performed 120 hr after the implantation of the first morphine pellet.

† $P < 0·05$ when compared to sham-operated rats.

time is a measure of the brain 5-HT turnover rate. The data listed in Table 4 show that this accumulation rate is equal in sham-operated and morphine-implanted rats.

Table 5 reports the values of brain tryptophan and brain 5-HT steady state concentrations and their specific activity after a pulse injection of [^{3}H]-tryptophan. The rate of the initial change of tryptophan and serotonin specific activity is equal in both groups of rats. Hence $k_{5\text{-HT}}$ and turnover rate are not affected by the implantation of morphine pellets.

Table 6 lists the values of plasma tyrosine and brain NE steady state concentrations and their specific activity. The plasma tyrosine concentrations are elevated in rats implanted with morphine, hence the value of the specific activity of plasma tyrosine 5 min after labeling is lower in morphine-implanted than in sham-operated rats. At later times, the specific activity of plasma tyrosine of morphine-implanted rats is equal to that of sham-operated rate. The turnover rate estimation based on the k_{NE} calculated from this value fails to show any difference between the two groups of rats.

DISCUSSION

In many experimental animals, morphine reduces brain concentrations of NE and DM.[13–16] In animals made tolerant to morphine, injections of this drug fail to deplete brain amines.[17] Since monoamine brain concentrations are maintained by continuous synthesis and metabolism, the focus of pharmacological studies of brain monoamines has been shifted almost completely from measurements of steady state levels of monoamine compartments to the study of the dynamic properties that maintain steady state conditions. In line with this shift of emphasis came the report by Way *et al.*[1] that morphine dependence is associated with increased turnover rate of brain 5-HT. By injecting intracisternally [^{14}C]-tyrosine at various times after morphine (60 mg/kg) and measuring accumulation of [^{14}C]-NE and [^{14}C]-DM 10 min after injection, Clouet and Ratner[18] have reported that morphine-tolerant rats incorporate more ^{14}C-tyrosine into brain catecholamines than naive animals given the same morphine dose. In these studies, hypothalamus and striatum were singled out as the areas where the effects of morphine were more marked. However, when the turnover of brain NE is measured[19] by calculating the rate of decline of [^{3}H]-NE injected intracisternally, the turnover of brain NE in tolerant rats is equal to that of naive rats. Moreover, no change in NE depletion after blockade of its biosynthesis was reported after chronic administration of morphine.[20] Our data on brain NE fail to substantiate the report of Clouet and Ratner[18] and agree with the findings of Neal[19] and Gunne *et al.*[20] Indeed the technique used by Clouet and Ratner[18] is inappropriate in more than one way: (1) a uniform distribution of the intracisternally injected [^{14}C]-tyrosine, if it occurs, is not an instantaneous phenomenon; (2) the initial specific activity of [^{14}C]-tyrosine may differ in brain of morphine-treated rats and in brain of untreated rats (see Table 6), but these authors do not measure tyrosine specific activity; (3) the conversion rate of tyrosine to catecholamines was not corrected for the actual values of the tyrosine specific activity. Because of this omission, it is impossible to compare the results obtained with morphine-treated naive rats, morphine-treated tolerant rats and untreated rats. Our results on brain 5-HT turnover rate also fail to substantiate the report by Way *et al.*[1] on mice. In rats, we repeated Way's[1] experiments with pargyline and with *p*-chlorophenylalanine. We could confirm neither the increased rate of 5-HT accumulation in morphine-dependent rats nor the decreased severity of the with-

drawal syndrome when 5-HT synthesis was inhibited. Since we used another animal species, we cannot claim that we failed to confirm the findings of Way *et al.*[1] However, in experiments carried out in collaboration with Cheney and Goldstein[21] of Stanford University, we could not confirm the finding of Way *et al.*[1] that morphine dependence increases brain 5-HT turnover in mice.[21]

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