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LEVELS AND DISTRIBUTION OF CENTRAL NERVOUS SYSTEM AMINES IN NORMAL AND MORPHINE-DEPENDENT MONKEYS*

M. SEGAL,† G. A. DENEAU and M. H. SEEVERS
Department of Pharmacology, University of Michigan, Ann Arbor

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Summary—The distribution of norepinephrine, dopamine and 5-hydroxytryptamine in various areas of the CNS of the normal monkey has been presented.

The central levels and distribution of norepinephrine, dopamine and 5-hydroxytryptamine were determined after single doses of morphine, during maintenance of physical dependence and following withdrawal from chronic administration of morphine. Small and non-stressful doses of morphine were employed to induce experimental physical dependence.

Alpha-methyl-DOPA did not qualitatively or quantitatively alter the abstinence syndrome.

Pretreatment with iproniazid did not prevent depression produced by single doses of morphine in the non-tolerant monkey.

The data observed in this study offer no support for the view that gross behavioral changes in the monkey produced by morphine or by its withdrawal after the development of physical dependence are induced by or may be correlated with changes in amine levels in the CNS.

CURRENT hypotheses which attempt to relate variations in behavioral and somatic responses to changes in catecholamine content of the CNS (e.g. HESS, CONNAMACHER, OZAKI and UNDENFRIEND, 1961) have inspired several investigators to search for such correlations during a cycle of chronic morphine administration and withdrawal (GUNNE, 1959, 1961; MAYNERT and KLINGMAN, 1962; KLINGMAN and MAYNERT, 1962; SHEN, FU-HSIUNG and WAY, 1970; EIDELBERG and SCHWARTZ, 1970; RETHY, SMITH and VILLARREAL, 1971; SMITH, VILLARREAL, BEDNARCZYK and SHELDON, 1970). Theoretically, this should be a good system to test this hypothesis since the range of somatic activity between the narcosis induced by a large single dose of morphine in the intact animal and the generalized hyperirritability of abstinence following chronic administration is about as great as possible short of the extremes of anesthesia and convulsions.

Previous investigations dealing with the rat, cat, rabbit and dog have several defects; morphine dosage was so large that the direct stimulant effect of the drug was necessarily an uncontrolled factor in these experiments; morphine administration was not sufficiently frequent to induce a stable dependent state and catecholamine determinations in rats were made on aliquots of the whole brains of several animals.

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†Supported by National Institute of Mental Health Fellowship No. MPD-11,959,C1. Present address: Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada.

In the present study, techniques designed to obviate these objections have been applied to the rhesus monkey (*Macaca mulatta*), a species which is quite similar to man in its response to single and repeated administration of morphine. Norepinephrine, dopamine and 5-hydroxytryptamine levels of selected parts of the CNS were determined after single doses of morphine, during maintenance of physical dependence and following withdrawal from chronic administration of morphine.

METHODS

All experiments were performed in the rhesus monkey (*Macaca mulatta*). Signs of depression following single subcutaneous injections of morphine and of excitation on withdrawal of morphine from dependent animals were graded and evaluated as previously described (SEEVERS, 1954; DENEAU, 1958). The animals were killed at the time of maximal drug effect.

Treatments. The experiments were divided into 4 groups on the basis of overt signs of behavior as related to the animals' levels of CNS excitability (Table 1). Group 1 consisted of 3 subgroups of 3 animals each whose behavior was essentially normal. These

TABLE 1. EXPERIMENTAL DESIGN

Groups	Subgroups*		
	a	b	c
Group 1 Behavioral controls (normal behavior)	Normals (untreated)	Nalorphine (2 mg/kg)	Physically dependent animals (3 mg/kg/6 hr)
Group 2 Morphine depression	Morphine (3 mg/kg)	Morphine (30 mg/kg)	—
Group 3 Withdrawal excitation	Abrupt (24 hr)	Abrupt (48 hr)	Nalorphine-induced
Group 4 Thebaine excitation	Thebaine (4 mg/kg)	—	—

*Three animals in each subgroup.

are represented in the tables as behavioral controls. The 3 subgroups were: (a) normals (no drug treatment), (b) animals killed 45 min following a single subcutaneous injection of 2 mg/kg nalorphine hydrochloride. This dose did not alter behavior in the monkey and served as a control for its use in precipitating withdrawal in dependent animals, (c) monkeys made physically dependent and tolerant to morphine sulfate on a chronic dosing schedule of 3 mg/kg every 6 hr and killed 90 min following the last injection of morphine (supported physically dependent animals). Group 2 consisted of 2 subgroups of 3 animals each in a state of behavioral depression. These were: (a) and (b) non-tolerant animals killed 90 min following single subcutaneous injections of 3 and 30 mg/kg morphine. Group 3 consisted of 3 subgroups of 3 animals each in a state of behavioral excitation. The 3 subgroups were (a) and (b) animals killed at 24 and 48 hr of abrupt withdrawal from morphine and (c) physically dependent animals killed at 45 min of withdrawal precipitated by the subcutaneous administration of 2 mg/kg nalorphine. Group 4 consisted

of 3 non-dependent animals in a state of mild excitation produced by the subcutaneous administration of 4 mg/kg thebaine hydrochloride. The animals were killed 30 min following the administration of thebaine. This agent does not possess the depressant properties of morphine and does not induce physical dependence on chronic administration.

Doses of all agents are expressed as their respective salts.

Preparation of animals. Just prior to death, 1 mg/kg of heparin sodium was injected i.v. to facilitate perfusion of the brain. The animal was then killed by the intracardiac injection of 1 g pentothal sodium. In the light of evidence that there can be considerable diurnal variation in brain amine levels, all animals were killed between 07:00 and 07:30 on the morning of each experiment. The brain was perfused with 200 ml of physiological saline at 200 mm Hg pressure in order to remove circulating amines and prevent their interference with the tissue assays. The calvarium was removed quickly and the brain along with a small section of cervical spinal cord was removed. The following parts were immediately dissected in a cold room at 4°C: cerebellum, dorsal thalamus, hypothalamus, caudate nucleus, part of the cervical spinal cord, medulla, pons, mid-brain (remaining part of the brain stem) and cerebral cortex. The time from death to the end of dissection was kept constant for all animals (50–70 min). Stabilizing the time-period was of the utmost importance at this point since loss of tissue amines occurs with the passage of time prior to extraction into an acid medium (EADE, 1961).

Tissue assay. An extract of each sample was prepared according to the method of MOORE and BRODY (1961) except that the final volume was 10 ml. Each extract was divided into two equal parts. One part was used for the determination of 5-hydroxytryptamine according to the method of BOGDANSKI, PLETSCHER, BRODIE and UDENFRIEND (1957); the second part was applied to columns of alumina (Fisher, acid-washed), eluted with acid and used for the determination of norepinephrine and dopamine. Norepinephrine was determined by the method of BERTLER, CARLSSON and ROSENGREN (1957) as modified by MOORE and BRODY (1961). Dopamine was determined by a modification of the method of CARLSSON and WALDECK (1958). Instead of irradiating the samples by means of a mercury lamp to enhance the development of fluorescence, the samples were allowed to stand for 1 hr at room temperature in a fluorescent-lit room before reading the fluorescence in an Aminco–Bowman spectrophotofluorometer.

Percentage recoveries ($\pm$S.D.) of norepinephrine, dopamine and 5-hydroxytryptamine simultaneously added to brain were: norepinephrine, 84 ± 6; dopamine, 67 ± 3; and 5-hydroxytryptamine, 106 ± 3. The amines were always analyzed in a single sample of brain. There was no cross interference by any of the amines in any of the assay procedures.

Statistical analyses. The data were analyzed statistically on an IBM 709 computer using the Analysis of Variance (FISHER, 1958) and the Duncan's Multiple Range Test (DUNCAN, 1955).

In vivo *studies with enzyme inhibitors, reserpine and morphine*

1. *Alpha-methyl-DOPA.* Alpha-methyl-DOPA was injected i.p. in a dose range of 50–600 mg/kg into 16 normal monkeys, i.v. in a dose range of 5–400 mg/kg to 6 normal monkeys and orally to 12 normal monkeys in a dose range of 100–600 mg/kg. The animals were observed and graded for 12–17 hr. Alpha-methyl-DOPA (400 mg/kg) was also injected i.v. into 4 physically dependent monkeys at 6, 8, 10 and 12 hr of abrupt withdrawal from morphine which were then observed for a further 6 hr.

2. *Iproniazid, reserpine, morphine.* Three normal monkeys were pretreated for 3 consecutive days with a total of 100 mg/kg iproniazid (isonicotinic acid-N-isopropyl hydrazide phosphate) subcutaneously. Twenty-four hours after the last injection of iproniazid, 1 animal was administered 0·5 mg/kg reserpine i.v., the second, 3 mg/kg morphine subcutaneously and the third served as a control. As controls for the pretreated animals, 2 untreated animals were administered 0·5 mg/kg reserpine and 3 mg/kg morphine respectively. All animals were graded for 8 hr following drug treatment.

RESULTS

Tables 2–4 summarize the levels and distribution of norepinephrine, dopamine and 5-hydroxytryptamine in the various areas of the monkey's CNS under 3 states of CNS excitability: overtly normal (behavioral controls), depression (morphine) and excitation (withdrawal). This type of presentation was selected since the numerous individual data were statistically insignificant. To indicate the degree of change we have selected the hypothalamus (Table 5). The hypothalamus was selected for illustrative purposes because of its likely involvement in the morphine abstinence syndrome during which a marked imbalance in autonomic function occurs and also in view of the fact that the relative level of amines found in this area reduce the methodological error.

1. *Normal amine distribution.* Norepinephrine was found in highest concentration in the hypothalamus (Table 2). The norepinephrine content of the hypothalamus was found to be approximately 3 times that of the dog and cat (VOGT, 1954). The norepinephrine levels in the other areas were similar to those reported for the dog and cat (VOGT, 1954). In the hypothalamus, they ranged from 2 to 4 μg/g, in the thalamus, medulla and mid-brain from 0·2 to 0·5 μg/g, in the pons and caudate nucleus from 0·1 to 0·2 μg/g and in the cerebellum, cerebral cortex and cervical spinal cord, less than 0·1 μg/g. Dopamine was most concentrated in the caudate nucleus (Table 3); small but variable amounts were present in all other areas. 5-Hydroxytryptamine, like norepinephrine, was most concentrated in the hypothalamus and mid-brain (Table 4). Other areas with a high content of 5-hydroxytryptamine were the thalamus, medulla, pons and caudate nucleus. Low levels were found in the cerebellum, cerebral cortex and cervical spinal cord.

2. *Physical dependence cycle.* Tables 2–4 show the distribution of the amines in the various areas of the monkey's CNS under different behavioral conditions. These tables demonstrate that only one statistically significant alteration occurred in the levels of distribution of these amines between the behavioral extremes of morphine depression and withdrawal excitation. Injections of 3–30 mg/kg morphine significantly ($P < 0{\cdot}01$) increased the cervical spinal cord level of norepinephrine (Table 2). Other trends towards altered amine levels with the various treatments were not significant.

During the mild behavioral excitation induced by the injection of 4 mg/kg thebaine, no significant alterations in CNS amine levels were observed (Table 5).

Marked variations from animal to animal obviated any correlations between dopamine and 5-hydroxytryptamine levels and treatments (Tables 3 and 4).

In vivo *studies with enzyme inhibitors, reserpine and morphine*

Even though no statistically significant alterations in CNS amine levels were produced with the various treatments further *in vivo* studies were performed in order to determine whether altered CNS amine levels were of biological significance in a cycle of physical

TABLE 2. DISTRIBUTION OF NOREPINEPHRINE IN VARIOUS AREAS OF THE CNS OF THE MONKEY UNDER NORMAL CONDITIONS AND DURING A CYCLE OF PHYSICAL DEPENDENCE TO MORPHINE

Areas / Treatment	Cerebellum	Thalamus	Hypo-thalamus	Caudate nucleus	Spinal cord	Medulla	Pons	Mid-brain	Cerebral cortex
Group 1*									
Behavioral controls	0·04† ±0·01‡	0·28 ±0·07	3·23 ±0·32	0·18 ±0·03	0·06 ±0·01	0·47 ±0·05	0·16 ±0·04	0·48 ±0·04	0·07 ±0·01
Group 2§									
Morphine depression	0·02 ±0·01	0·30 ±0·15	3·19 ±0·44	0·15 ±0·03	0·15¶ ±0·01	0·59 ±0·04	0·18 ±0·03	0·65 ±0·04	0·05 ±0·01
Group 3**									
Withdrawal excitation	0·03 ±0·01	0·19 ±0·07	2·11 ±0·16	0·14 ±0·03	0·04 ±0·01	0·37 ±0·05	0·07 ±0·03	0·50 ±0·06	0·10 ±0·02

*Mean of 9 values (3 normal animals, 3 injected s.c. with 2 mg/kg nalorphine and 3 physically dependent animals).
†All values expressed as μg/g.
‡S.E.M.
§Mean of 6 values (3 animals injected s.c. with 3 mg/kg morphine and 3 with 30 mg/kg morphine).
¶Statistically significant ($P < 0·01$).
**Mean of 9 values (3 withdrawals at 24 hr, 3 at 48 hr and 3 following nalorphine-precipitated withdrawal).

TABLE 3. DISTRIBUTION OF DOPAMINE IN VARIOUS AREAS OF THE CNS OF THE MONKEY UNDER NORMAL CONDITIONS AND DURING A CYCLE OF PHYSICAL DEPENDENCE TO MORPHINE

Area / Treatment	Cerebellum	Thalamus	Hypo-thalamus	Caudate nucleus	Spinal cord	Medulla	Pons	Mid-brain	Cerebral cortex
Group 1*									
Behavioral controls	0·05† ±0·20‡	0·42 ±0·13	0·48 ±0·18	6·12 ±0·38	0·50 ±0·29	0·17 ±0·08	0·19 ±0·15	0·13 ±0·04	0·10 ±0·05
Group 2§									
Morphine depression	0·01 ±0·004	0·20 ±0·13	0·65 ±0·27	6·95 ±0·22	0·18 ±0·18	0·03 ±0·02	0·07 ±0·05	0·21 ±0·12	0·05 ±0·02
Group 3¶									
Withdrawal excitation	0·03 ±0·01	0·41 ±0·13	0·78 ±0·24	7·38 ±0·43	0·18 ±0·10	0·05 ±0·03	0·37 ±0·15	0·15 ±0·06	0·08 ±0·03

*Mean of 9 values (3 normal animals, 3 injected s.c. with 2 mg/kg nalorphine and 3 physically dependent animals).
†All values expressed as μg/g.
‡S.E.M.
§Mean of 6 values (3 animals injected with 3 mg/kg morphine and 3 with 30 mg/kg morphine).
¶Mean of 9 values (3 withdrawals at 24 hr, 3 at 48 hr and 3 following nalorphine-precipitated withdrawal).

TABLE 4. DISTRIBUTION OF 5-HYDROXYTRYPTAMINE IN VARIOUS AREAS OF THE CNS OF THE MONKEY UNDER NORMAL CONDITIONS AND DURING A CYCLE OF PHYSICAL DEPENDENCE TO MORPHINE

Area / Treatments	Cerebellum	Thalamus	Hypo-thalamus	Caudate nucleus	Spinal cord	Medulla	Pons	Mid-brain	Cerebral cortex
Group 1*									
Behavioral controls	0·09† ±0·02‡	0·82 ±0·13	1·06 ±0·16	0·64 ±0·08	0·33 ±0·07	0·88 ±0·13	0·69 ±0·10	1·03 ±0·11	0·22 ±0·03
Group 2§									
Morphine depression	0·12 ±0·01	1·28 ±0·25	1·34 ±0·22	0·65 ±0·10	0·41 ±0·06	1·21 ±0·28	0·64 ±0·10	1·46 ±0·30	0·29 ±0·06
Group 3¶									
Withdrawal excitation	0·10 ±0·01	1·20 ±0·16	1·00 ±0·13	0·65 ±0·07	0·36 ±0·03	0·92 ±0·10	0·55 ±0·10	1·22 ±0·15	0·25 ±0·03

*Mean of 9 values (3 normal animals, 3 injected s.c. with 2 mg/kg nalorphine and 3 physically dependent animals).
†All values expressed as μg/g.
‡S.E.M.
§Mean of 6 values (3 animals injected s.c. with 3 mg/kg morphine and 3 with 30 mg/kg morphine).
¶Mean of 9 values (3 withdrawals at 24 hr, 3 at 48 hr and 3 following nalorphine-precipitated withdrawal).

TABLE 5. MEAN HYPOTHALAMIC AMINE LEVELS IN A CYCLE OF PHYSICAL DEPENDENCE TO MORPHINE

Treatments / Amines	Group 1			Group 2		Group 3			Group 4
	No drug treatment	Acute Nalorphine (2 mg/kg)	Physically dependent (3 mg/kg/ 6 hr)	Acute morphine (3 mg/kg)	Acute morphine (30 mg/kg)	24-hr withdrawal	48-hr withdrawal	Nalorphine withdrawal	Thebaine (4 mg/kg)
Norepinephrine	3·36*† (2·97–3·60)‡	3·02 (1·73–4·77)	2·89 (2·46–2·63)	2·70 (2·10–3·10)	3·67 (2·87–5·27)	2·24 (1·57–2·94)	1·86 (1·35–2·32)	2·24 (1·88–2·47)	4·35 (3·19–5·67)
Dopamine	1·03 (0·57–1·42)	0·42 (0·00–0·86)	0·00 (0·00–0·00)	0·59 (0·00–1·77)	0·72 (0·57–0·90	0·64 (0·00–1·20)	1·40 (0·00–2·12)	0·31 (0·00–0·93)	3·38 (1·00–7·50)
5-Hydroxytryptamine	1·00 (0·40–1·65)	1·36 (1·23–1·43)	0·72 (0·30–1·33)	0·95 (0·64–1·20)	1·73 (1·27–2·13)	1·15 (0·91–1·40)	0·97 (0·28–1·35)	0·88 (0·60–1·30)	1·33 (0·70–2·35)

*All values represent the mean of 3 animals.
†All values expressed as μg/g.
‡Range of 3 animals.

dependence to morphine. Agents known to affect the level of CNS excitability and to alter CNS amine levels were used.

1. *Alpha-methyl-DOPA.* Alpha-methyl-DOPA did not produce significant signs of sedation in normal monkeys and did not qualitatively or quantitatively alter the abstinence syndrome.

2. *Iproniazid, reserpine, morphine.* Pretreatment of the monkey for 3 days with a total of 100 mg/kg of the monoamine oxidase inhibitor, iproniazid, did not consistently prevent the depression produced by an i.v. injection of 0·5 mg/kg reserpine and did not qualitatively or quantitatively alter the depression produced by a subcutaneous dose of 3 mg/kg morphine in the nontolerant monkey.

DISCUSSION

The important question to be answered in the present study was: could the extremes of behavioral signs as observed during acute administration of morphine (depression) and withdrawal of morphine from dependent animals (excitation) be correlated with an altered level of distribution of CNS amines?

VOGT (1954) showed that the hypothalamus of dogs and cats contained the highest concentration of norepinephrine and that high doses of morphine (30–60 mg/kg) reduced the levels in cats but not in dogs. GUNNE (1959) reported that 30 mg/kg morphine decreased levels of rat brain norepinephrine, but doses of 60–90 mg/kg morphine increased these levels. From these studies it would appear that doses of morphine which produce overt behavioral depression depress brain norepinephrine levels while doses which produce overt stimulation increase these levels. Although they did not report the behavioral effects of the doses they employed, MAYNERT and KLINGMAN (1962) reported depressed levels of brain catecholamines with all doses (60–150 mg/kg to rats and up to 200 mg/kg to dogs). The higher doses which they used in rats and dogs are known to produce mixed signs of overt depression and stimulation.

Contrary to the findings of GUNNE (1959, 1961), SLOAN, BROOKS, EISENMAN and MARTIN (1963) reported that brain catecholamine levels showed a statistically significant increase over saline controls in their experimental design where rats received twice daily subcutaneous injections of morphine sulfate starting at a dose of 5 mg/kg/day with increases up to 400 mg/kg/day over a 40-day period. The present data are at variance with these findings since small, and, for the monkey, moderately depressant doses of morphine (3 and 30 mg/kg) did not alter CNS catecholamine levels significantly except in the cervical spinal cord in which both doses produced an increase. There is no correlation between the effect of morphine on brain catecholamine levels and on CNS excitability among the various species studied thus far.

GUNNE (1959) reported that rat brain norepinephrine levels decreased during withdrawal from morphine following a 3-week chronic dosing schedule of 20 mg/kg increasing to 200 mg/kg per dose, injected i.p., twice daily. MAYNERT and KLINGMAN (1962) confirmed this finding during withdrawal in dogs and rabbits but observed no changes during withdrawal of morphine from rats. In the present study, the catecholamine levels did not change significantly during withdrawal from morphine. The inconsistency of the data in these several studies fail to support the view that changes in brain norepinephrine levels are causally related to CNS hyperexcitability associated with the morphine abstinence syndrome.

Differences observed between the results of this study and those of other investigators might be due to: (1) the species chosen as experimental animal; (2) the dose and schedule employed to induce physical dependence to morphine and (3) the analysis of individual CNS areas as opposed to total or pooled brain analyses. These differences can be further qualified as follows: (1) Many factors influence drug metabolism (CONNEY and BURNS, 1962) COSTA, GESSA, HIRSCH, KUNTZMAN and BRODIE (1962) reported that iproniazid pretreatment prevented reserpine depression in mice. As presently reported, this phenomenon did not occur consistently in the monkey. Variation between species as well as within a species must be considered when interpreting data. (2) When doses of morphine in the range of 30–200 mg/kg are injected once or twice daily to produce physical dependence, the animals are in a sequence of stressing conditions due to alternating daily periods of morphine excitation and withdrawal. Doses in the higher range produce primary excitation and severe secondary depression. It is impossible under these conditions to establish a relatively steady state which corresponds to that which exists in man or the monkey when small doses are administered at frequent intervals. In the monkey, the maximal stabilizing period between injections of small doses of morphine (3 mg/kg) has been shown to be 6 hr (DENEAU, 1958). The animals show only mild signs of depression after injection and do not show withdrawal hyperirritability between injections. We believe the changes in catecholamine levels observed in small animals with large infrequent dosage of morphine to be a reflection of experimental design rather than a true manifestation of behavioral effects related to physical dependence. (3) A detailed study of the distribution of CNS amines under various conditions (e.g. physical dependence cycle) offers more opportunity to detect meaningful changes than analyses of total or pooled brain samples.

It should be stressed that negative results obtained with methods designed to measure only tissue content, like those described here, do not exclude amines from final consideration in attempts to correlate biochemical change with behavior since such methods offer no information concerning rates of formation, release or degradation.

Recently, SHEN *et al.* (1970) and SMITH *et al.* (1970) reported increased turnover rates of 5-hydroxytryptamine and catecholamines in morphine-tolerant and -dependent mice and rats. Although the conclusions reached relate to an association between amine turnover and the morphine physical dependence cycle, no causal relationships were established. Further experimentation by MARSHALL and GRAHAME-SMITH (1970) failed to confirm the SHEN *et al.* (1970) increase in 5-hydroxytryptamine turnover and although MARUYAMA, HAYASHI and TAKEMORI (1970) found no causal relation between brain 5-hydroxytryptamine metabolism and physical dependence, they concluded that some relation between tolerance and 5-hydroxytryptamine turnover may exist. CHENEY, GOLDSTEIN, ALGERI and COSTA (1971) and ALGERI and COSTA (1971), however, have recently furnished evidence that serotonin turnover in the brain is not related to morphine-tolerance and -dependence. There is as yet no final agreement that altered amine kinetics are a causal factor in morphine physical dependence.

Altered dopamine levels observed during the various stages of CNS hyperirritability encountered in a cycle of physical dependence to morphine might suggest a role in this syndrome apart from its being a precursor of norepinephrine. *In vivo* studies with α-methyl DOPA showed that this agent, which prevents the decarboxylation of DOPA and depresses the central dopamine levels in various species ranging from mouse to man (HESS, 1954; OATES, GILLESPIE, UDENFRIEND and SJOERDSMA, 1960; PORTER, TOTARO and LEIBY, 1961) decreased the intensity of the morphine abstinence syndrome in mice (HUIDOBRO,

CONTRERAS and CROXATTO, 1963) and altered at least some of the behavioral effects of administered morphine (EIDELBERG and SCHWARTZ, 1970), did not qualitatively or quantitatively alter the abstinence syndrome in the monkey. Assuming that α-methyl DOPA affects CNS amine levels in monkeys, as in other species, dopamine is probably not causally related to the development of physical dependence to morphine.

Iproniazid is known to alter CNS amine levels in mice, rats, rabbits, cats, dogs and man (SPECTOR, PROCKOP, SHORE and BRODIE, 1958; LEROY and DESCHAEPDRYVER, 1961; GAMROT, ROSENGREN and GOTTFRIES, 1962) to affect the level of CNS excitability in these same species (SPECTOR *et al.*, 1958; PARE, 1959) and to lessen or prevent reserpine depression and locomotor effects in mice (SMITH and DEWS, 1962; COSTA *et al.*, 1962; MARSHALL and GRAHAME-SMITH, 1970). Pretreatment of nontolerant monkeys with iproniazid did not prevent depression produced by the acute injection of morphine. Assuming that iproniazid affects monoamines and monoamine oxidase in monkeys as in other species, it appears that the depressant action of morphine is not dependent on the levels of these amines.

We confirm the results of others (BRODIE, SHORE and PLETSCHER, 1956; MAYNERT, KLINGMAN and HIDEKO, 1962; SLOAN *et al.*, 1963) that 5-hydroxytryptamine is not altered during the various stages of CNS excitability encountered during a cycle of physical dependence to morphine.

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