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EFFECTS OF MORPHINE, *IN VITRO* AND *IN VIVO*, ON TYROSINE HYDROXYLASE ACTIVITY IN RAT BRAIN*

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Abstract—The effect of morphine on the synthesis of catecholamines was determined in rat brain. In agreement with other studies, morphine produced a dose-dependent increase in the biosynthesis of the catecholamines. To assess whether morphine might enhance the synthesis of norepinephrine and dopamine by a direct chemical interaction with tyrosine hydroxylase, the rate-limiting step in their biosynthesis, the effects of the narcotic on the activity of the enzyme were determined in several regions of rat brain. Morphine, *in vitro*, from 10^{-8} to 10^{-2} M had no effect on the activity of tyrosine hydroxylase in any region examined. Moreover, morphine (10^{-5} and 10^{-3} M) had no effect on the apparent K_m of tyrosine hydroxylase for either substrate or cofactor (6-7-dimethyl-5,6,7,8-tetrahydropterine). In addition, morphine (10^{-5} and 10^{-3} M) failed to block the inhibition of tyrosine hydroxylase, *in vitro*, by norepinephrine and dopamine in the hypothalamus and caudate respectively. The effects of morphine treatment, *in vivo*, on enzyme activity were also examined. The results of these studies indicated that acute injections of morphine had no effect on tyrosine hydroxylase activity, *in vitro*, indicating that the drug did not alter the level of an endogenous activator or inhibitor of tyrosine hydroxylase. Further studies indicated that development of tolerance to and physical dependence on morphine was not associated with an increase in the activity of brain tyrosine hydroxylase activity. The results of these studies suggest that morphine does not enhance the biosynthesis of catecholamines by a direct effect of tyrosine hydroxylase and that tolerance to the narcotic is not characterized by an induction of this enzyme.

RECENTLY, Smith *et al.*[1,2] and Clouet and Ratner[3] demonstrated that morphine increases the rate of synthesis of catecholamines in the mouse and rat brain. In subsequent studies, Smith *et al.*[1,2] have shown that the effect of morphine on the biosynthesis of norepinephrine and dopamine is blocked by the narcotic antagonist naloxone and that tolerance and cross-tolerance with other narcotics develop to this effect. On the basis of these findings, these investigators concluded that catecholamine-containing neurons in brain may be fundamentally involved in at least some of the effects of morphine on central nervous system (CNS) function.

Although the mechanism whereby the narcotics enhance the synthesis of catecholamines in brain is unknown, a drug-induced activation of tyrosine hydroxylase, the rate-limiting step in the biosynthesis of the catecholamines,[4–6] seems likely based on the considerable evidence which has accumulated demonstrating the primary, and perhaps exclusive, role of this enzyme in regulating metabolic flux along the catecholamine biosynthetic pathway.[7–10] At present, however, it is only a matter of conjecture

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whether narcotics alter the activity of tyrosine hydroxylase by a direct interaction with the enzyme or whether some indirect mechanism may be involved.

The possibility that tolerance to the narcotics may be associated with an "induction" of tyrosine hydroxylase has also been suggested by several investigators.[3,11] This speculation arises, at least in large part, from data implicating the catecholamines in the CNS effects of the narcotics[2,12,13] and the findings that inhibitors of protein synthesis, such as puromycin and cyclohexamide, block the development of tolerance to and physical dependence on morphine.[14,15] In support of this hypothesis, Reis *et al.*[11] have reported that chronic morphine treatment results in an increase in the activity of tyrosine hydroxylase in the caudate nucleus of the rat brain. This observation, however, has recently been disputed by Smith *et al.*[2] who found that the rate of synthesis of catecholamines in the brains of morphine-tolerant mice was no different from that observed in control animals, suggesting that tolerance to the narcotics was not characterized by an "induction" of tyrosine hydroxylase.

Because of the considerable speculation regarding the role of tyrosine hydroxylase in both the acute and chronic effects of the narcotics, the present experiments were carried out to examine the effects of morphine, *in vitro* and *in vivo*, particularly during the development of tolerance and physical dependence, on tyrosine hydroxylase activity in the midbrain-pons, hypothalamus and caudate nucleus of the rat brain. These three regions were selected for study since the effects of morphine on catecholamine biosynthesis are quite marked in these areas[2,3] and a good deal of evidence suggests that each may be involved in regulating a number of brain functions affected by the narcotics.

METHODS

Chemicals and drugs. Morphine sulfate was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. DL-Norepinephrine-bitartrate was purchased from the Sigma Chemical Corp., St. Louis, Mo. and 6-7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_4$) was obtained from CalBiochem Co., Los Angeles, Calif. L-Tyrosine (^{14}C, uniformly labeled) was obtained from the New England Nuclear Co., Boston, Mass. All other chemicals were of reagent grade and were purchased from the Fisher Chemical Co., St. Louis, Mo. Naloxone was a generous gift of Endo Laboratories, Garden City, N.Y.

Morphine pellets were formulated by the Private Formulae Co., St. Louis, Mo., and consisted of 75 mg of morphine base, 75 mg of microcrystalline cellulose, 0·75 mg of fumed silicon dioxide and 1·5 mg of magnesium stearate.[16] Placebo pellets were also formulated but contained an equivalent amount of lactose in place of the morphine.

Animals and tissue preparation. Male Holtzman rats weighing between 100 and 150 g were used in all the experiments. At the completion of all studies, the rats were decapitated and the brains were rapidly removed (< 45 sec) and frozen on dry ice. They were then stored at −80° until dissected. At the time of dissection, the brains were thawed on ice and the midbrain-pons, hypothalamus and caudate nucleus were dissected out at 4° according to a protocol completely described elsewhere.[17] The specimens were then frozen on dry ice, weighed at −17° and homogenized in 4 vol. of ice-cold water. The homogenates were then stored at −80° until the tyrosine hydroxylase assays (see below) were performed.

Drug treatments. For studies of the effects of morphine *in vitro*, tissue samples were obtained from untreated control animals (usually pooled from three to five animals)

as described above. Morphine sulfate was dissolved in the acetate buffer used for the tyrosine hydroxylase assays and was added directly to the incubation mixture. All concentrations of morphine in the final incubation volume were calculated on the basis of the free base.

For the studies of the acute effects of systemically administered morphine, *in vivo*, on tyrosine hydroxylase activity, rats were injected subcutaneously with either saline (0·9 per cent) or various concentrations of morphine sulfate dissolved in saline. After 45 min, the time at which the peak analgesic effect occurs,[18] the rats were decapitated, and the brains were removed and dissected as described above. They were then weighed and homogenized, and tyrosine hydroxylase activity was determined.

For studies of the effects of chronic morphine treatment on tyrosine hydroxylase activity, rats were implanted with either morphine or lactose pellets, subcutaneously. The rats were briefly anesthetized in an ether–ethylchloride (1:1) vapor chamber and a small incision was made on the back of the neck between the shoulder blades on midline. The pellets were then inserted 1 cm from the incision which was closed with a wound clip. The pellet implantation technique and the behavioral and pharmacologic aspects of this procedure have been completely described elsewhere.[18] The rats were then killed at various intervals for up to 7 days after the initial pellet implantation. At the appropriate time the rats were decapitated, the brains were removed and tissue samples were obtained as described above.

Assay of tyrosine hydroxylase. Tyrosine hydroxylase activity was measured in the brain regions by a micro-modification of the method of Nagatsu *et al.*[19] which we have completely described elsewhere.[17] Enzyme activity was measured in approximately 2 mg wet weight of brain. The tissue extracts were incubated in a buffer substrate mixture consisting of: 0·1 mM L-tyrosine, containing approx. $2{\cdot}0 \times 10^5$ cpm of purified (3,5-^{3}H)L-tyrosine; 0·5 mM $Fe(NH_4)_2(SO_4)_26 . H_2O$; 0·67 mM $DMPH_4$; 0·12 M 2-mercaptoethanol; 0·2 M sodium acetate buffer (pH = 6·0) and, when appropriate, concentrations of morphine (in the final incubation mixture) of 10^{-8} to 10^{-2} M. The incubation mixture consisted of 110 μl of the buffer-substrate and 10 μl of brain extract (1 → 5 homogenate). Blanks consisted of 10 μl of water or boiled brain extract. All ingredients were added at 4° and the samples were then incubated at 37° for 30 min. The reaction was terminated by immersing the tubes in an ice water bath and by adding 400 μl of 5% (w/v) trichloroacetic acid. The mixture was then centrifuged at 2000 rev/min for 10 min and the supernatant fluids were transferred to columns (disposable Pasteur pipets) packed with 5 cm Dowex 50-W (H^+ form, 200–400 mesh). The incubation tubes were washed in succession with 1000 and 500 μl of water and the contents were transferred to the columns. The combined eluates were collected in scintillation vials and 15 ml of Bray's[20] solution was added. The samples were then counted in a Packard Tri-Carb liquid scintillation counter.

Synthesis of catecholamines in brain. To determine whether morphine enhanced catecholamine biosynthesis in the rat brain, rats were injected with either 0 (saline), 10, 20 or 30 mg/kg of morphine sulfate, subcutaneously. Thirty minutes later, ^{14}C-tyrosine (50 μCi/kg) was injected in the tail vein. The rats were then killed 45 min later, the time at which the maximum incorporation of ^{14}C-tyrosine into catecholamines occurs;[2,3] their brains were rapidly removed and frozen on dry ice. The whole brain was homogenized in 10 ml of 5% trichloroacetic acid and centrifuged at 15,000 rev/min for 30 min. The supernatant fluids were saved and divided into two aliquots,

one for the determination of the ^{14}C label incorporated into brain catecholamines (75 per cent of the total supernatant fluids) and the other for the determination of the specific activity of the tyrosine precursor pool (25 per cent of the supernatant fluids). The ^{14}C label incorporated into the catecholamines as well as the specific activity of the tyrosine precursor were determined by methods described by Smith *et al.*[2] Endogenous tyrosine levels in whole brain were determined by the method of Waalkes and Udenfriend.[21] The recovery of total catecholamines in all studies reported in this paper averaged between 95 and 98 per cent.

RESULTS

Effects of morphine on catecholamine biosynthesis. The effect of morphine on the synthesis of catecholamines in whole rat brain is depicted in Table 1. As shown in this table, morphine markedly increased the synthesis of ^{14}C-catecholamines from ^{14}C-tyrosine over control levels (0 morphine, Table 1) at each dose examined. Moreover,

TABLE 1. EFFECTS OF MORPHINE ON ^{14}C-CATECHOLAMINE BIOSYNTHESIS FROM ^{14}C-TYROSINE, *in vivo*, IN WHOLE RAT BRAIN*

Dose of morphine (mg/kg)	^{14}C-catecholamines (dis/min/g wet wt)
0	3546 (± 492·5)
10	4789 (± 293·2)
20	6891 (± 411·4)†
30	8312 (± 573·2)‡

* See text for experimental details.
† $P < 0{\cdot}05$.
‡ $P < 0{\cdot}01$.

this effect was dose related with the greatest degree of enhancement (more than two-fold greater than the control levels) of catecholamine biosynthesis occurring at the 30 mg/kg dose level. There were no differences in the specific activity of the tyrosine precursor pool in brain between any of the morphine-injected groups and the control group.

Effects of morphine, in vitro, *on tyrosine hydroxylase activity.* The effects of a range of morphine concentrations, *in vitro* (from 10^{-8} to 10^{-3} M in the final incubation mixture), on tyrosine hydroxylase activity are shown in Table 2. In the experiments

TABLE 2. EFFECTS OF MORPHINE (10^{-8} TO 10^{-3} M IN THE FINAL INCUBATION MIXTURE) ON TYROSINE HYDROXYLASE ACTIVITY IN THE CAUDATE NUCLEUS AND WHOLE RAT BRAIN*

	Concentrations of morphine (M)						
Region	0	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Caudate nucleus	1477·7 (± 72·9)	1484·9 (± 55·5)	1479·1 (± 60·9)	1542·7 (± 40·9)	1484·7 (± 86·3)	1459·9 (± 44·3)	1528·7 (± 62·8)
Whole brain	94·46 (± 4·2)	103·6 (± 6·4)	94·0 (± 6·4)	101·0 (± 7·9)	89·1 (± 3·1)	86·8 (± 6·5)	88·3 (± 4·3)

* Tyrosine hydroxylase activity is expressed as nmoles of tyrosine oxidized/g of tissue wet wt/hr. Values represent the mean (±S.E.M.) of three experiments.

summarized in this table, the standard incubation mixture used in the tyrosine hydroxylase assays was employed (Methods) and data have been presented for the effects of morphine on tyrosine hydroxylase activity in both whole brain and caudate nucleus homogenates. As can be seen in this table, none of the concentrations of morphine employed in these experiments affected the activity of tyrosine hydroxylase in either of the tissue homogenates. Additional experiments utilizing homogenates derived from hypothalamus and midbrain-pons provided results analogous to those shown in Table 2.

Since the experiments described above were performed under assay conditions providing maximal activation of tyrosine hydroxylase, the possibility that morphine might alter the K_m or V_{max} of tyrosine hydroxylase for either the tyrosine substrate or pteridine cofactor may not have been detected. Therefore, experiments were conducted to determine the apparent K_m and V_{max} of tyrosine hydroxylase for both substrate and cofactor with and without added morphine in the incubation mixture. The tissue homogenate used in these studies was derived from the caudate nucleus of untreated control animals. The incubation mixture was identical to that described in the Methods with the exception that either the substrate or cofactor concentration was varied.

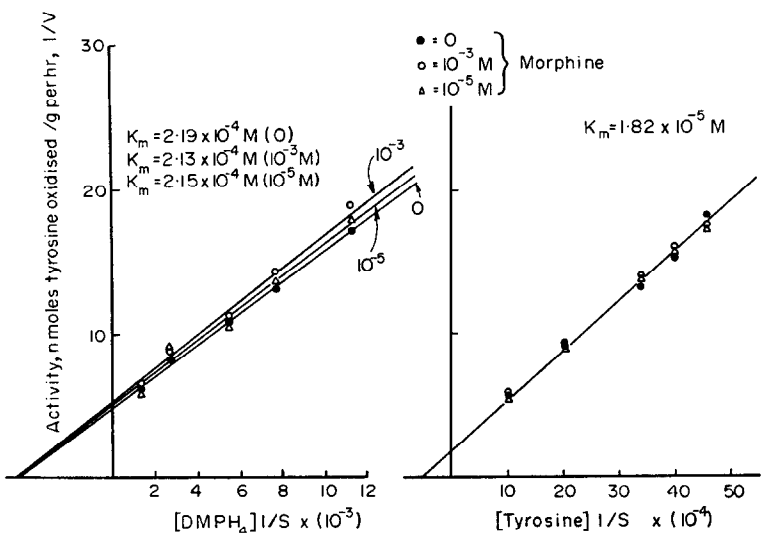

FIG. 1. Double-reciprocal plots of tyrosine hydroxylase activity (nmoles tyrosine oxidized/g/hr) as a function of the concentration of $DMPH_4$ (left panel) and the concentration of tyrosine (right panel) in the incubation mixture, with (10^{-3} M and 10^{-5} M) and without (0) added morphine. The incubation mixture used in these assays was identical to that described in Methods with the exception that substrate and cofactor concentrations were varied as illustrated in the figure. Each point represents the mean of three separate experiments. The line drawn through the individual points represents the best-fit line as determined by a least-squares analysis. See text for further details.

Figure 1 (left panel) shows the double-reciprocal plot of tyrosine hydroxylase activity, with and without added morphine (10^{-5} and 10^{-3} M), as a function of the concentration of $DMPH_4$ in the incubation mixture. As shown in this figure, neither concentration of morphine produced a significant change in either the apparent K_m or V_{max} of tyrosine hydroxylase for $DMPH_4$. The slight degree of variation seen in this figure can be entirely attributed to normal experimental error ($\pm$ 8–11 per cent).

Figure 1 (right panel) also shows the double-reciprocal plot of tyrosine hydroxylase activity, with and without morphine, as a function of the concentration of the tyrosine substrate in the incubation mixture. A single line has been drawn through all the points in this figure since individual least-squares analyses for each group (i.e. 0, 10^{-3} M and 10^{-5} M) revealed essentially the same best-fit line. As can be seen in this figure, neither concentration of morphine (10^{-5} and 10^{-3} M) changed the apparent K_m or V_{max} of tyrosine hydroxylase for the tyrosine substrate. Other experiments utilizing homogenates derived from either whole brain or hypothalamus also failed to establish any effect of morphine on the affinity of tyrosine hydroxylase for $DMPH_4$ or tyrosine.

Since tyrosine hydroxylase is rate-limiting and its activity appears to be determined by end-product feedback inhibition,[6,22] we examined whether morphine might block the inhibition of tyrosine hydroxylase by norepinephrine and dopamine, *in vitro*. In these experiments, we examined the effects of morphine on the inhibition of tyrosine hydroxylase by norepinephrine in the hypothalamus and by dopamine in the caudate nucleus, since these candidate transmitters are thought to be selectively localized in these two regions. The results of these studies are presented in Table 3. As shown in

TABLE 3. EFFECTS OF MORPHINE (10^{-8} to 10^{-3} M IN THE FINAL INCUBATION MIXTURE) ON NOREPINEPHRINE INHIBITION OF TYROSINE HYDROXYLASE ACTIVITY IN HYPOTHALAMUS AND DOPAMINE INHIBITION IN THE CAUDATE NUCLEUS OF THE RAT BRAIN*

	Concentrations of morphine (M)						
Catecholamine	0	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Norepinephrine (10^{-4} M)	37·94 (± 6·84)	36·33 (± 6·29)	38·02 (± 3·05)	46·79 (± 7·34)	31·86 (± 5·48)	40·53 (± 3·12)	48·96 (± 1·57)
Dopamine (10^{-4} M)	78·88 (± 1·50)	79·92 (± 3·49)	79·15 (± 2·04)	78·88 (± 2·21)	79·83 (± 0·32)	83·59 (± 1·32)	83·19 (± 1·33)

* Figures given are per cent (%) inhibition of enzyme activity as compared to maximal enzyme activity in the absence of added end product. All concentrations of catecholamine and morphine were calculated as the free base. Assay procedure was the same as that described in Methods. Each value represents the mean (± S.E.M.) of four experiments.

this table, norepinephrine, at 10^{-4} M, produced approximately 40 per cent inhibition of tyrosine hydroxylase activity in the hypothalamus. (It should be noted with regard to norepinephrine inhibition of tyrosine hydroxylase activity in the hypothalamus, that a much greater degree of inhibition could have been achieved by lowering the level of $DMPH_4$ in the incubation mixture, since norepinephrine appears to compete with this cofactor for an active site on tyrosine hydroxylase. However, in the present experiments any greater degree of inhibition of tyrosine hydroxylase activity in the hypothalamus by norepinephrine would have resulted in values too low to be accurately determined.) As shown in Table 3, morphine concentrations from 10^{-8} to 10^{-3} M did not appreciably alter the inhibition of tyrosine hydroxylase by norepinephrine in the hypothalamus. In the case of dopamine inhibition of tyrosine hydroxylase activity in the caudate, a concentration of 10^{-4} M produced around an 80 per cent inhibition of tyrosine hydroxylase. However, in agreement with the data presented

above, morphine concentrations of 10^{-5} and 10^{-3} M did not prevent the inhibition of tyrosine hydroxylase activity by dopamine in the caudate nucleus (Table 3).

Effects of morphine, in vivo, *on tyrosine hydroxylase*. The effects of chronic morphine treatment, leading to the development of a marked degree of tolerance and physical dependence, on tyrosine hydroxylase activity in the caudate nucleus, midbrain-pons and hypothalamus are shown in Fig. 2. In this figure, tyrosine hydroxylase, expressed

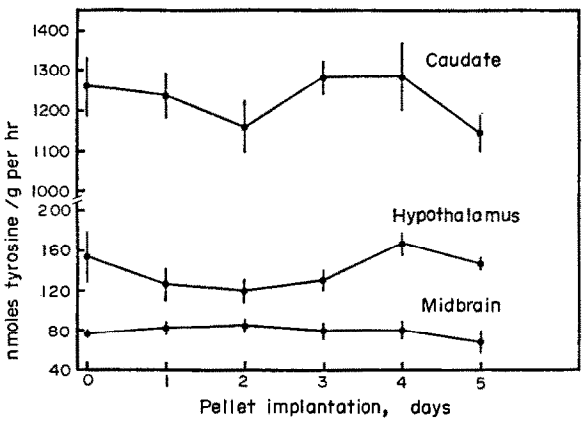

FIG. 2. The activity of tyrosine hydroxylase (nmoles tyrosine oxidized/g/hr) in caudate nucleus, hypothalamus and midbrain–pons as a function of the days after morphine pellet implantation. Each point represents the mean (± S.E.M.) of six rats.

as nmoles of tyrosine oxidized/g tissue wet weight/hr, has been plotted as function of the days after the initial morphine pellet implantation. As can be seen in this figure, there was no change in the activity of tyrosine hydroxylase in any of the three regions examined throughout the period of morphine pellet implantation. Particularly noteworthy in this figure is the lack of any change in tyrosine hydroxylase activity when both tolerance to and physical dependence on the narcotic reached their peak levels (i.e. by day 3 of pellet implantation). To assess the possibility that chronic morphine treatment might produce an alteration in the K_m or V_{max} of tyrosine hydroxylase for either substrate or cofactor without a concurrent induction of the enzyme, the activity of tyrosine hydroxylase as a function of substrate and cofactor concentration was determined in morphine-tolerant and non-tolerant animals. In agreement with studies of the effects of morphine *in vitro* on tyrosine hydroxylase, chronic morphine treatment did not result in any change in the apparent K_m or V_{max} of the enzyme for either tyrosine or $DMPH_4$ when compared to naïve, non-tolerant rats.

To evaluate whether morphine might elevate the level of an endogenous activator or, alternatively, depress the level of an endogenous inhibitor of tyrosine hydroxylase, rats were injected intraperitoneally with various concentrations of morphine and killed 45 min later. Tyrosine hydroxylase activity was then determined, *in vitro*, in homogenates of caudate nucleus, hypothalamus and midbrain–pons of the treated animals. The results of these studies are summarized in Table 4. As shown in this table, acute doses of morphine, ranging from 5–20 mg/kg, did not significantly alter the level of tyrosine hydroxylase activity over control values (0 morphine, Table 4) in any of the

TABLE 4. EFFECTS OF ACUTE DOSES OF MORPHINE ON TYROSINE HYDROXYLASE ACTIVITY IN CAUDATE NUCLEUS, HYPOTHALAMUS AND MIDBRAIN-PONS OF RAT BRAIN*

Region	Morphine (mg/kg)				
	0	5	7·5	10·0	20·0
Caudate nucleus	1330·9 (± 70·2)	1447·9 (± 83·6)	1434·1 (± 52·78)	1310·5 (± 72·3)	1304·9 (± 87·6)
Hypothalamus	171·5 (± 10·9)	200·8 (± 28·83)	185·7 (± 16·8)	150·1 (± 21·1)	178·1 (± 13·8)
Midbrain–pons	115·6 (± 5·6)	113·9 (± 14·2)	110·7 (± 6·6)	122·4 (± 5·7)	116·5 (± 5·0)

* Tyrosine hydroxylase activity is expressed as nmoles of tyrosine oxidized/g of tissue wet wt/hr. Each value represents the mean (±S.E.M.) tyrosine hydroxylase activity for six rats.

regions examined. Subsequent pilot studies utilizing a wider range of morphine concentrations ranging from 2·5 to 35 mg/kg (which is a lethal dose in many of our rats) provided results entirely consistent with those reported above.

DISCUSSION

The results of the studies described in this paper indicate that morphine, *in vitro*, and acute and chronic treatment, *in vivo*, does not appreciably alter the activity of tyrosine hydroxylase, the rate-limiting step in the biosynthesis of the catecholamines.[4,5] Moreover, our results argue against the possibility that tolerance to morphine may be associated with an induction of the tyrosine hydroxylase enzyme, since there was little or no change in the activity of the enzyme at any time during the period of pellet implantation, even though, as we have previously demonstrated,[18] this procedure produces an extremely high degree of tolerance to and physical dependence on the narcotic. Our failure to demonstrate an "induction" of tyrosine hydroxylase during the development of tolerance to morphine is consistent with the observation of Smith *et al.*[2] that the rate of synthesis of catecholamines in the morphine-tolerant mouse was no different from that observed in non-tolerant controls. Our data, however, and those of Smith *et al.*[2] do not agree with a paper by Reis *et al.*[11] in which they reported a substantial increase in the activity of tyrosine hydroxylase in the caudate nucleus of the morphine-tolerant rat over non-tolerant control levels. We are unable, at this time, to explain the discrepancy between our results and those of the latter investigators.

Although the present results suggest that the development of tolerance to the narcotics is not associated with a generalized increase in tyrosine hydroxylase activity or enzyme amount, we cannot rule out the possibility that changes do occur in enzyme activities in certain discrete areas or, perhaps, cells in the regions we examined which we were unable to detect. However, since the samples taken for analysis in these studies averaged 10 mg or less in weight, it seems probable that we would have detected at least some effect on the enzyme if any significant change had occurred. A final answer to this question is not possible at this time utilizing existing assay procedures and must await further refinements in technique, probably the development of immunofluorescence assays. However, on the basis of the present data we can conclude that tolerance to the narcotics is not characterized by a marked increase in either the activity

and/or amount of tyrosine hydroxylase in either whole brain or in a number of anatomically well-defined brain regions.

The data presented in this paper indicate that morphine, *in vitro*, does not alter the affinity of tyrosine hydroxylase for either substrate or cofactor. Moreover, our data indicate that morphine does not interfere with the inhibition of tyrosine hydroxylase by the catecholamines, the mechanism presently thought to be most critically involved in the regulation of metabolic flux along the catecholamine pathway.[7,8] On the basis of the above data, it seems unlikely that morphine, *in vivo*, exerts a direct action on tyrosine hydroxylase in producing an enhanced synthesis of catecholamines. Rather, our data appear to be more consistent with the position that the increased biosynthesis of norepinephrine and dopamine observed after acute morphine treatment is due to an indirect action of the drug on catecholamine-containing neurons. Although numerous indirect mechanisms could be postulated, one possible way in which morphine could produce this effect would be by blocking the action of the catecholamines at adrenergic receptors in brain or, perhaps, in the peripheral nervous system. This possibility is suggested by a number of reports by Dairman and coworkers[8,10,23] in which they demonstrated that α-adrenergic blockade produced a greatly enhanced rate of catecholamine biosynthesis in the heart, adrenals and brain of the rat. In preliminary work in our laboratory, we have begun to examine the interaction of morphine with a number of α- and β-adrenergic blockers. As an initial step in this direction, we are presently examining the interaction of morphine with the α-blocker, phenoxybenzamine. Our data thus far indicate that pretreatment with phenoxybenzamine (either 3 or 22 hr) results in a slight degree of analgesia, as assayed by the hot-plate method,[18,24] and a greatly exaggerated analgetic and lethal response to very low doses of morphine in the rat. Although these preliminary data certainly do not establish that the effects of morphine on brain function are mediated by adrenergic mechanisms, the similar effects of α-blockers and morphine on catecholamine biosynthesis and the preliminary data just described are consistent with this interpretation and warrant a good deal of further examination.

REFERENCES

1. C. B. Smith, J. E. Villarreal, J. H. Bednarczyk and M. I. Sheldon, *Science, N.Y.* **170,** 1106 (1970).
2. C. B. Smith, M. I. Sheldon, J. H. Bednarzyk and J. E. Villarreal, *J. Pharm. exp. Ther.* **180,** 547 (1972).
3. D. H. Clouet and M. Ratner, *Science, N.Y.* **168,** 854 (1970).
4. M. Levitt, S. Spector, A. Sjoerdsma and S. Udenfriend, *J. Pharmac. exp. Ther.* **184,** 1 (1965).
5. S. Udenfriend, P. Zaltzman-Nirenberg, R. Gordon and S. Spector, *Molec. Pharmac.* **2,** 95 (1966).
6. S. Spector, R. Gordon, A. Sjoerdsma and S. Udenfriend, *Molec. Pharmac.* **3,** 549 (1967).
7. G. C. Sedvall and I. J. Kopin, *Biochem. Pharmac.* **16,** 39 (1967).
8. W. Dairman, R. Gordon, S. Spector, A. Sjoerdsma and S. Udenfriend, *Molec. Pharmac.* **4,** 457 (1968).
9. R. A. Mueller, H. Thoenen and J. Axelrod, *J. Pharmac. exp. Ther.* **169,** 74 (1969).
10. B. L. Bigelow, W. Dairman, H. Weil-Malherbe and S. Udenfriend, *Molec. Pharmac.* **5,** 565 (1969).
11. D. J. Reis, P. Hess and E. A. Azmitia, Jr., *Brain Res., Osaka* **20,** 309 (1970).
12. L. M. Gunne, *Acta physiol. scand.* **58** (suppl. 204), 5 (1963).
13. C. R. Rethy, C. B. Smith and J. E. Villarreal, *J. Pharmac. exp. Ther.* **176,** 472 (1971).
14. E. L. Way, H. H. Loh and F. Shen, *Science, N.Y.* **162,** 1290 (1968).
15. A. A. Smith, M. Karmin and J. Gavitt, *Biochem. Pharmac.* **15,** 1877 (1966).
16. R. D. Gibson and J. F. Tingstad *J. pharm. Sci.* **59,** 426 (1970).

17. T. J. CICERO, L. G. SHARPE, E. ROBINS and S. S. GROTE, *J. Neurochem.* **19,** 2241 (1972).
18. T. J. CICERO and E. R. MEYER, *J. Pharmac. exp. Ther.* **184,** 404 (1973).
19. T. NAGATSU, M. LEVITT and S. UDENFRIEND, *Analyt. Biochem.* **9,** 122 (1964).
20. G. A. BRAY, *Analyt. Biochem.* **1,** 279 (1960).
21. T. P. WAALKES and S. UDENFRIEND, *J. Lab. clin. Med.* **50,** 733 (1957).
22. S. UDENFRIEND, P. ZALTZMAN-NIRENBERG and T. NAGATSU, *Biochem. Pharmac.* **14,** 837 (1965).
23. W. DAIRMAN and S. UDENFRIEND, *Molec. Pharmac.* **6,** 350 (1970).
24. T. JOHANNESSON and L. A. WOODS, *Acta pharmac. tox.* **21,** 381 (1964).