Narcotic Drugs: Effects on the Serotonin Biosynthetic Systems of the Brain

Abstract. *The effects of short- and long-term administration of morphine on the activity of two measurable forms of rat brain tryptophan hydroxylase were studied. Morphine administration produced an immediate decrease and a long-term increase in the nerve ending (particulate) enzyme activity but did not change the cell body (soluble) enzyme activity. Cocaine administration demonstrated a short-term decrease in measurable nerve ending enzyme activity that was due to the inhibition of the high affinity uptake (the Michaelis constant, K_m, is 10^{-5} molar) of trytophan, the serotonin precursor. Cocaine did not affect the low affinity uptake ($K_m = 10^{-3}$ molar) of tryptophan. Both the uptake of the precursor and the enzyme activity appeared to be drug-sensitive regulatory processes in the biosynthesis of serotonin.*

Numerous studies have been concerned with relating the short-term and long-term effects of morphine to its influence on the serotonergic pathways in the central nervous system. The drug-induced changes have generally involved alterations in measures of turnover of the amine. Brain concentrations of serotonin (5-hydroxytryptamine) have not been shown to change during the development of tolerance or physical dependence (*1*). The findings of several groups have been somewhat in conflict. Serotonin turnover has been shown to increase in response to morphine (*2*) or to be unaffected (*3*) depending on the experimental variables. Tryptophan hydroxylase, the rate-limiting enzyme in the biosynthesis of serotonin, has been shown by some investigators to increase after long-term administration of morphine (*4*) and not to change by others (*5*).

We report here the effects of short-term and long-term morphine administration on the activity of two measurable forms of tryptophan hydroxylase, soluble (cell body) and particulate (nerve ending) enzyme, and on the uptake of tryptophan into synaptosomes prepared from an area rich in serotonin nerve endings. We have shown that the particulate or nerve ending form appears to be regulated by the uptake of substrate, the soluble form by the amount or activity of the enzyme itself (*6*). We now demonstrate differential response of these two forms of enzyme to narcotic drugs.

For studies of the short-term effects of morphine, male Sprague-Dawley rats (180 to 200 g) were injected subcutaneously with morphine sulfate (10 mg/kg), 2 to 3 hours prior to decapitation, and each control rat received an equal volume (equivolume) of saline. The long-term administration of morphine was carried out by the implantation (subcutaneously) without anesthesia of a 75-mg pellet of morphine (provided by Dr. E. Way) 5 days prior to decapitation. Control rats were treated to the same operative procedure without pellet implantation.

Tryptophan hydroxylase was determined in two brain regions: (i) soluble enzyme from an area rich in serotonin cell bodies, the midbrain; and (ii) particulate enzyme from the septal area, a region with many serotonin nerve endings (*7*). After the animals were decapitated the brain regions were dissected free and were chilled on ice. The midbrain was homogenized in either 2 m*M* potassium phosphate (*p*H 7.0) or 0.32*M* sucrose by means of a Thomas glass-Teflon homogenizer with a 0.010-cm clearance. The homogenates were centrifuged in a Sorvall RC-2B centrifuge at 45,000*g* for 20 minutes. The supernatants, which contained 80 to 90 percent of the measurable midbrain tryptophan hydroxylase activity, served as a representative source for soluble (cell body) enzyme assays. The septal area was homogenized in 0.32*M* sucrose and the crude mitochondrial or P_2 fraction was prepared as described by Gray and Whittaker (*8*). This fraction contained 85 percent of the tryptophan hydroxylase activity of the septal region and served as a representative enzyme source for studies of nerve ending enzyme.

Soluble and particulate tryptophan hydroxylase was measured by modifications (*6*) of the methods of Ichiyama *et al.* (*9*). L-[1-^{14}C]Tryptophan (12 μc/μmole) was used as substrate. The product, $^{14}CO_2$, was liberated by the action of L-amino acid decarboxylase on 5-hydroxytryptophan. We measured aromatic amino acid decarboxylase in the same regions as described above by the method of Christenson *et al.* (*10*) using L-[1-^{14}C]dopa (53 μc/μm) as substrate. The liberated $^{14}CO_2$ in both assays was trapped in NCS and counted in a liquid scintillation spectrometer by means of an external standard quench correction.

The uptake of substrate by synaptosomes was studied with L-[3-^{14}C]tryptophan (29 μc/μmole) as the substrate and septal P_2 pellet as the source of serotonin synaptosomes. The incubation medium was 0.1*M* tris (*p*H 8.1) or modified Ringer phosphate (*p*H 7.5) containing 10 m*M* glucose and 10^{-5}*M* L-[3-^{14}C]tryptophan. When substrate concentrations were varied, this preparation demonstrated uptake K_m's of 10^{-3}*M* and 10^{-5}*M* for the radioactive tryptophan substrate that were glucose and temperature dependent. That this effect was uptake rather than binding was suggested by the finding that hypotonic shock of the synaptosomes abolished all tryptophan uptake. Whether accrued radioactivity represented net increases in total intrasynaptosomal substrate and product, or facilitated exchange of exogenous tryptophan, is yet indeterminate. All labeled amino acids were obtained from New England Nuclear.

The short-term effects of morphine sulfate (administered 2 to 3 hours prior to decapitation) were a small but significant decrease in the activity of septal particulate tryptophan hydroxylase but not in the measurable midbrain soluble enzyme activity (Fig. 1). In contrast, long-term exposure to morphine by pellet implantation for 5 days resulted in a significant increase in the activity of nerve ending enzyme, but not in the cell body preparation. At no time could a significant change in the specific activity of aromatic amino acid decarboxylase be demonstrated in either area. Cocaine, studied for its short-term effects, demonstrated a small but significant decrease in nerve ending but not cell body enzyme activity.

Figure 2 demonstrates the effects of in vitro morphine sulfate and cocaine hydrochloride on the activities of soluble tryptophan hydroxylase, particulate tryptophan hydroxylase, and the uptake of tryptophan by septal synaptosomes. In vitro as well as in vivo (short-term administration), both morphine and cocaine cause an apparent decrease in hydroxylating activity of the particulate enzyme (Fig. 1). However, this apparently similar effect of the two drugs appears to be mediated by two different mechanisms. (i) Morphine inhibits the soluble enzyme in reasonable intra-

synaptosomal concentrations ($10^{-4}M$) but has no effect on the uptake of tryptophan by the particulate fraction, and (ii) cocaine has no effect on the soluble enzyme within a wide range of concentrations but inhibits tryptophan uptake into the synaptosomal biosynthetic unit. The relatively high morphine concentration of 1×10^{-4} to $5 \times 10^{-4}M$ necessary to inhibit the soluble enzyme in vitro may represent the circumstances in vivo in that experiments have shown that morphine is taken up and concentrated by synaptosomes (*11*). Cocaine, in contrast, has no demonstrable effect on soluble tryptophan hydroxylase although it impairs the high affinity uptake of tryptophan, and by this mechanism results in the decrease of particulate tryptophan hydroxylase activity. We have some evidence that this cocaine inhibition is noncompetitive for the substrate. Cocaine, on the other hand, had no effect on the low affinity uptake of tryptophan.

Our studies demonstrate that morphine and cocaine do affect certain variables of potential influence on the serotonergic biosynthetic system under the experimental conditions used both in vivo and in vitro. Further, they provide examples in vitro of two possible neurobiological mechanisms by which the serotonin biosynthetic system by way of its rate-limiting enzyme, tryptophan hydroxylase, might be altered by narcotic drugs. In addition, the significance of determining regional differences in neurotransmitter biosynthetic enzyme activity in both measurable enzyme forms rather than whole brain or single region homogenate or soluble enzyme has been exemplified.

The differential response to pharmacological manipulations of various regions of the serotonergic system is consistent with previous studies by Costa *et al.* (*12*) showing that forebrain but not midbrain serotonin turnover was altered by in vivo drug administration to rats. In addition, when rat forebrain but not midbrain serotonin was depleted by a median forebrain lesion, there was shown to be a +0.8 correlation between the pain threshold and the amount of forebrain, but not midbrain sertonin (*13*). Our results may explain the incompatability of previous studies of the response of brain tryptophan hydroxylase activity to morphine. For example, Azmitia and co-workers (*4*), using whole midbrain homogenate as the enzyme source, demonstrated a small increase in enzyme activity in response to long-term morphine treatment. The small size of the increase could be due to the relatively small proportions of serotonin nerve endings in this midbrain preparation. Areas such as the septal region with higher density of nerve endings (*6, 7, 14*) show much greater increases (see Fig. 1). Schechter *et al.* (*5*) reported no change in tryptophan hydroxylase activity after morphine pellet implantation using a synthetic pterin cofactor [2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine ($DMPH_4$)] dependent fraction prepared from whole mouse brain. As only soluble enzyme could be dependent on exogenous $DMPH_4$ since nerve endings do not take up this cofactor (*6, 15*) this is consistent with our finding that the

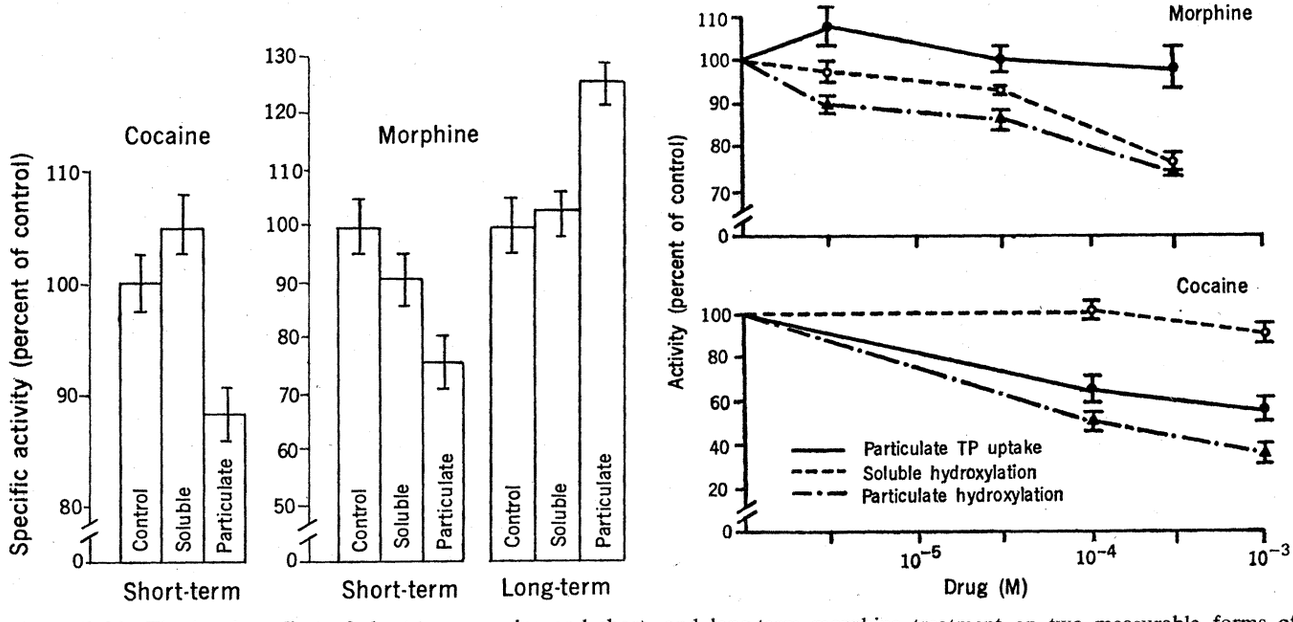

Fig. 1 (left). The in vivo effect of short-term cocaine and short- and long-term morphine treatment on two measurable forms of regional brain tryptophan hydroxylase in the rat. For short-term treatment, cocaine (30 mg/kg) and morphine (10 mg/kg) were administered subcutaneously 2 to 3 hours prior to decapitation of the animals. For long-term exposure to morphine a 75-mg morphine tablet was implanted for 5 days. Substrate concentration was $2 \times 10^{-5}M$ for the measurement of enzyme activity. The amount of control soluble activity was 80 to 100 pmole mg^{-1} hr^{-1} and of control particulate activity was 100 to 120 pmole mg^{-1} hr^{-1}. The number of animals per group was 15 to 18. In all cases particulate enzyme activity is significantly reduced or increased ($P = .05$) from its control level. Levels of statistical significance were determined by the Mann-Whitney U test (*17*). Fig. 2 (right). The in vitro effects of morphine and cocaine on the uptake of tryptophan (*TP*) by the septal crude, mitochondrial pellet, the hydroxylation of tryptophan by the midbrain soluble preparation and the septal crude mitochondrial preparation. Substrate concentration for the measurements of enzyme activity and uptake of tryptophan was $10^{-5}M$. Control velocity values for the uptake were 450 to 500 pmole mg^{-1} 10 min^{-1}; for soluble hydroxylation, 100 pmole mg^{-1} hr^{-1}; and for the particulate hydroxylation, 125 pmole mg^{-1} 45 min^{-1}. Each series was obtained with regional preparations pooled from five rats; each experiment was repeated at least three times. In response to morphine, both measurable forms of tryptophan hydroxylase activity are reduced and significantly different ($P = .05$) from their control values. In contrast, in response to cocaine, tryptophan uptake and particulate hydroxylation are reduced significantly ($P = .05$) from their control values. Statistical significance was determined by the Mann-Whitney U test (*17*).

soluble $DMPH_4$-dependent form of tryptophan hydroxylase does not manifest a change in activity in response to long-term exposure to morphine.

The presence of two measurable forms of tryptophan hydroxylase in brain, their varying regional distribution, their differing modes of regulation, and their differential response to pharmacological agents may explain some of the conflicts seen in the literature relating the action of morphine to its effects on the serotonin biosynthetic system. It is possible that some of these differences could be resolved if drug dose, time after administration, and region could be more uniform and specific. It appears that the late increases in nerve ending tryptophan hydroxylase after the short-term inhibition of enzyme activity by morphine is consonant with the findings of our program of research, which demonstrates compensatory changes in presynaptic neurotransmitter biosynthetic enzyme activity after drug-induced changes in synaptic function (*16*).

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Deficits in Feeding Behavior after Intraventricular Injection of 6-Hydroxydopamine in Rats

Abstract. *Intraventricular injections of 6-hydroxydopamine produced 95 percent depletion of telencephalic norepinephrine and 62 percent depletion of striatal dopamine in rats. Treated rats maintained body weight at subnormal levels and failed to increase food intake in response to a short-term decrease in glucose utilization. After treatment with the monoamine oxidase inhibitor pargyline, 6-hydroxydopamine produced no further norepinephrine depletion but increased the dopamine depletion to 95 percent and produced complete aphagia. These effects are comparable to events that follow bilateral electrolytic lesions of the lateral hypothalamus.*

There is increasing evidence that brain catecholamines are involved in the control of food intake in rats. For example, lateral hypothalamic lesions produce aphagia in rats (*1, 2*) and have been reported to cause widespread depletion of brain norepinephrine and dopamine (*3*). Furthermore, aphagia has been observed after extrahypothalamic electrolytic or electrothermal lesions, or knife cuts, of a nigrostriatal pathway traversing the hypothalamus (*4, 5*) that includes a large dopamine-containing fiber system (*6*). Similar observations also have been made after intracerebral injections of 6-hydroxydopamine (6-HDA) along this pathway (*5, 7*), a procedure thought to cause a relatively specific degeneration of catecholamine-containing neurons (*8, 9*).

Intraventricular injections of 6-HDA also produce long-lasting depletions of brain catecholamines and should cause considerably less nonspecific damage than any of the above treatments. However, as yet little or no apparent effect of this treatment on feeding behavior has been observed (*9*). These observations are not consistent with evidence supporting an involvement of catecholamines in the control of feeding behavior. Therefore, we have determined whether subtle effects on this behavior may have been produced by the 6-HDA treatments but eluded observation by previous investigators because they were not specifically examined. We now report that 6-HDA administered intraventricularly can produce specific deficits in the feeding behavior of rats which parallel those of rats with lateral hypothalamic damage. Furthermore, these behavioral deficits are associated with substantial decreases in the concentrations of brain catecholamines in both groups of animals.

Male albino rats of the Sprague-Dawley strain (Zivic-Miller, Pittsburgh), weighing 175 to 225 g at the beginning of the experiment, were housed and tested in individual wire-mesh cages. They were allowed free access to Purina Chow pellets and tap water unless otherwise noted. Using ether as anesthesia, we injected 20 μl of either 200 μg of 6-hydroxydopamine hydrobromide (*10*) ($n = 10$) or the vehicle (0.9 percent NaCl, 0.1 percent ascorbic acid) ($n = 6$) into the cerebrospinal fluid by way of the left lateral ventricle. Ten days later the same injection was delivered by way of the right lateral ventricle of each rat (*11*).

Food intake decreased by 27 to 46 percent during the first 24 to 36 hours after each administration of 6-HDA and then returned toward normal. Testing was begun 3 weeks after the last injection. For three successive days, food intake in the home cage (with water present) was measured every hour for 7 hours (9:30 a.m. until 4:30 p.m.). On the fourth day, after the first hour of testing, each rat was injected intraperitoneally with 750 mg of 2-deoxy-D-glucose per kilogram of body weight to induce short-term decreases in glucose utilization (glucoprivation) (*12*). Food intake was monitored hourly for the following 6 hours. Control rats increased their feeding within the first hour after injection and consumed