Amphetamine-induced inhibition of tyrosine hydroxylation in homogenates of rat corpus striatum

A remarkable effect of amphetamine is that it appears to produce inhibition of catecholamine-synthesis uniquely in the corpus striatum but not in other areas of the brain such as the cerebral cortex or hypothalamus (Javoy, Hamon & Glowinski, 1970; Harris & Roth, 1973). The mechanisms underlying this unique inhibitory effect on tyrosine hydroxylation are not well understood and we have attempted to elucidate the mechanisms involved.

Tyrosine hydroxylase (TH) activity was assayed by the rate of generation of $^{14}CO_2$ from L-[1-^{14}C]dopa newly synthesized from L-[1-^{14}C]tyrosine by a modification of a tryptophan-5-monooxygenase assay developed for isolated nerve endings by Karobath (1972) which utilizes only the endogenous pteridine cofactor present in the tissue. Thus, corpora striata of young adult male Sprague-Dawley rats were dissected free over ice and homogenized in 10 vol of 0·32 M sucrose by a motor-driven Teflon pestle modified to produce 0·25 mm clearance in a glass Potter-Elvehjem homogenizer. Homogenates were centrifuged at 1000 × *g* for 15 min to sediment nuclei and debris and several 50 μl portions of the supernatant (equivalent to 5 mg wet wt of the striatum) containing synaptosomes and other cellular components were added to test tubes containing 175 μl of a physiological buffer solution at pH 6·7 (Harris & Roth, 1973) and in most experiments the reaction was started with 25 μl of a solution containing 0·25 μCi of L-[1-^{14}C]tyrosine (50 Ci mmol^{-1}, New England Nuclear). We found that the rate of production of $^{14}CO_2$ was half-saturated at 3·5 μM tyrosine; assays were routinely conducted at 20 μM substrate for 20 min in 250 μl total volume and yielded radioactivity 10 × above a boiled tissue "blank". The reaction was linear with the amount of tissue and with time for at least 30 min; it did not proceed with D-tyrosine as substrate and it was inhibited by 3-iodotyrosine (IC_{50} <5 μM) when L-[^{14}C]tyrosine was substrate, but not with L-[1-^{14}C]dopa. Dopamine inhibited the reaction strongly (IC_{50} <1 μM). The results were compared with an assay in which [^{14}C]dopa newly formed from L-[U-^{14}C]tyrosine (20 μM) in the presence of an inhibitor of dopa-decarboxylase (NSD-1055, 0·1 mM) was recovered by alumina column chromatography; the two methods gave closely similar results. The results support the conclusion that this coupled hydroxylase-decarboxylase $^{14}CO_2$ assay is a valid measure of the rate of tyrosine hydroxylation which proceeds in the presence of natural endogenous cofactors.

When 200 g male Sprague-Dawley rats were given (+)-amphetamine SO_4 (5 mg kg^{-1}, i.p.), TH activity in homogenates of corpora striata remained unchanged for 15–30 min, followed by an inhibition (30% at 30 min) (Table 1) which increased with time (50% at 120 min) and had returned to normal within 20 h. This treatment was found to have no effect on the uptake of tyrosine or the decarboxylation of L-[1-^{14}C]-dopa. When amphetamine was incubated with striatal tissue *in vitro* a slight acceleration of catechol synthesis occurred (18% at 10 μM), while the hydroxylated metabolites *p*-OH-amphetamine or *p*-OH-norephedrine inhibited tyrosine hydroxylation *in vitro* (30% at 5 μM). Nevertheless, it seems unlikely that the hydroxylated metabolites of amphetamine are responsible for the observed inhibition of catecholamine-synthesis following *in vivo* treatment. Thus, pretreatment with a large dose of the microsomal oxidase inhibitor SKF-525 A (40 mg kg^{-1}, i.p.), which prevents *p*-hydroxylation of amphetamine, failed to block the inhibitory action of amphetamine. Furthermore, the effects with guinea-pig, which is virtually incapable of *p*-hydroxylating amphetamine (Smith & Dring, 1970) were similar to those in the rat brain. Also, large systemic doses (100 mg kg^{-1}, i.p.) of the hydroxylated metabolites of amphetamine

Table 1. *Effects of drugs or nigro-striatal lesions on tyrosine hydroxylase activity in rat corpus striatum.*

Condition	% of control ± s.e. (n = 5 animals)
(+)-Amphetamine SO_4 (5 mg kg^{-1})	69 ± 2*
Chlorpromazine·HCl (10 mg kg^{-1})	100 ± 5
Chlorpromazine·HCl (5 min before (+)-amphetamine SO_4)	92 ± 4
Bicuculline (2·5 mg kg^{-1})	100 ± 5
Bicuculline (30 min before (+)-amphetamine)	100 ± 5
Nigro-striatal lesions	70 ± 2*

Rats were given the drugs in saline or saline alone (controls) intraperitoneally and corpora striata were removed after death 30 min later, homogenized and tyrosine hydroxylase activity was estimated in a crude synaptosomal preparation. Other animals were given unilateral (left) electrothermic lesions to the nigro-striatal tract 30 min before death and results were compared with the intact (right)side. Mean control activity ±s.e. = 20·5 ± 0·40 nmol tyrosine hydroxylated g^{-1} wet wt h^{-1}. (*): Indicates significant difference by Student's *t*-test ($P < 0{\cdot}01$) of the difference between mean enzyme activity in control *vs* treated rats, or by paired *t*-test in the case of unilaterally lesioned animals, comparing the lesioned and intact sides.

had no effect of tyrosine hydroxylation in the rat corpus striatum, possibly due to their poor entry through the blood-brain barrier.

An alternative possibility was that the inhibition of catechol-synthesis in the striatum following amphetamine might be related to functional changes in the nigro-striatal pathway. When unilateral radio-frequency electrothermic lesions were made under halothane-anaesthesia according to the method of Hökfelt & Ungerstedt (1969) in the midbrain substantia nigra, the activity of striatal TH on the side of the lesion decreased (Table 1) as early as 30 min later in comparison with the contralateral intact tissue. This decrease was presumably due to interruption of neuronal activity in the ascending nigro-striatal pathway, and it remained at 60–80% of control activity for 18–24 h; thereafter there was a rapid fall in enzyme activity to 10–20% of control, probably due to anterograde degenerative changes in striatal dopamine terminals. This finding suggests that decreased neurophysiological activity in nigro-striatal fibres may lead to decreased rates of dopamine synthesis, probably by increased local accumulations of it in the striatal terminals (Smith, 1965; Andén, Bédard & Ungerstedt, 1972, Harris & Roth, 1973), and further that the effects of amphetamine may be mediated by its reported ability to decrease the rate of firing of nigral cells (Bunney, Walters & others, 1973), an inhibition also produced by electrical stimulation of the caudate nucleus (Precht & Yoshida, 1971), and to produce an increase in striatal levels of dopamine (Smith, 1965; Harris & Roth, 1973). The basis of the rapidly occurring rise of dopamine levels in the striatum following an acute nigro-striatal lesion is not completely clear. Accumulation by the failure of release induced by nerve impulses is one likely mechanism, although the occurrence of a transient increase (several minutes) in the rate of dopamine synthesis by an unknown mechanism has also been suggested (Kehr, Carlsson & others, 1972). In addition to amphetamine it has also been reported that apomorphine or ET-495 (trivastal)—putative dopamine-receptor agonists —can also reduce the turnover or synthesis of striatal dopamine *in vivo* or following their administration *in vivo* by a mechanism blocked by neuroleptic drugs (Andén, Rubensson & others, 1967; Corrodi, Farnebo & others, 1972; Kehr & others, 1972; Goldstein, Anagnoste & Shirron, 1973). Nevertheless, both dopamine agonists may have local presynaptic actions as they can decrease its synthesis in striatal slices *in vitro* (Goldstein & others, 1973).

Pretreatment with chlorpromazine, a proposed blocker of dopamine receptors in the striatum, prevented the inhibitory effect of amphetamine on tyrosine hydroxylation

(Table 1). On the other hand, reserpine (5 mg kg^{-1}, i.p.), a neuroleptic drug which appears not to interfere with dopamine receptors, failed to do this. The proposed blockers of γ-aminobutyric acid (GABA) receptors, bicuculline (Table 1) and picrotoxin (2·5 mg kg^{-1}, i.p.) also blocked the action of amphetamine, while they themselves did not alter TH activity.

The effect of amphetamine on tyrosine hydroxylation does not appear to be due to hyperthermia as it occurred at doses which did not raise rectal temperature and it was not blocked by lowering body temperature after the administration of amphetamine by exposing the animals to a 10° environment or reproduced by elevating rectal temperature by exposure to a 48° environment.

In conclusion, the present results suggest that the unique inhibitory effects of *in vivo* administration of amphetamine on TH activity in striatal synaptosomes may be mediated by decreased neuronal activity in ascending nigro-striatal dopamine neurons. This could lead to local accumulations of dopamine within a small strategic (reserpine-resistant) pool within striatal terminals resulting in end-product inhibition of TH. This effect appeared to be interrupted by the blockade of striatal dopamine receptors, and by blockers of GABA-receptors (Table 1), suggesting that descending GABA-neurons (Kim, Bak & others, 1971; Precht & Yoshida, 1971; McGeer, Fibiger & others 1973) may be involved in a neurophysiological feed-back regulation of activity in the nigro-striatal pathway.

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