*RELEASE OF NEWLY SYNTHESIZED DOPAMINE FROM DOPAMINE-CONTAINING TERMINALS IN THE STRIATUM OF THE RAT**

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Abstract.—The extraneuronal spontaneous efflux of ^{3}H-dopamine synthesized endogenously in dopamine terminals has been demonstrated *in vitro*, by collecting the ^{3}H amine in superfusates of isolated striatum of the rat previously incubated with ^{3}H-tyrosine. The ^{3}H-dopamine newly synthesized appears to be preferentially released. Furthermore, amphetamine and a monoamine oxidase inhibitor (Catron) markedly increased the release of ^{3}H-dopamine. This effect was also observed with substances normally contained in some striatal neurons: acetylcholine and 5-hydroxytryptamine.

Introduction.—The *in vivo* release of dopamine, a putative neurotransmitter found in high concentrations in the numerous varicosities of specific terminals of the caudate nucleus, has been shown in the cat in only a few studies. On the basis of experiments in which a push-pull cannula was used to "superfuse" an area of the caudate nucleus, McLennan[1] reported that a resting-state release of dopamine could be observed; its release was enhanced by electrical stimulation of the nucleus centromedianus. Under similar experimental conditions, McKenzie and Szerb[2] could identify the released amine precisely only when very high doses of D-amphetamine were simultaneously applied locally. Portig and co-workers[3] have also demonstrated a pharmacologically inducible release of dopamine from the caudate nucleus; they detected the amine in ventricular perfusates following injections of *d*-tubocurarine. They also claimed that dopamine could be released by electrical stimulation of the substantia nigra, although that was not a consistent finding. Baldessarini and Kopin[4] have shown that ^{3}H-norepinephrine, taken up and stored in brain slices, can be released by field stimulation. They also reported similar results with ^{3}H-dopamine.

Many observations, made both *in vivo* and *in vitro*, have revealed that radioactive catecholamines, taken up in brain tissue, label the endogenous amine stores[5], but the possibility remains that small quantities of these ^{3}H-amines may not be specifically localized. If so, this could have a serious implication to release studies because, as shown in peripheral adrenergic nerves, the amount of amine released and detectable extraneuronally appears to be quite small when compared to the intraneuronal amine content.[6]

To avoid this possible objection to the use of exogenous ^{3}H-dopamine and also to avoid the relatively low sensitivity of the fluorimetric dopamine assay, we have attempted to demonstrate and to study the release of ^{3}H-dopamine, previously synthesized endogenously in dopamine-containing terminals from ^{3}H-tyrosine of high specific activity. Under our experimental conditions, the mini-

mal amount of ^{3}H-dopamine detectable is about 5 to 10 pg (1000 times more sensitive than the dopamine fluorimetric assay). Furthermore, since tyrosine hydroxylase is exclusively distributed in catecholamine-containing neurons, the ^{3}H-dopamine formed from ^{3}H-tyrosine is specifically localized. Finally, this approach permits an analysis of the relationships between synthesis and release mechanisms.

With this experimental approach, the resting and pharmacologically inducible release of dopamine has been demonstrated *in vitro* in the isolated rat striatum and *in vivo* in the caudate nucleus of the curarized cat. In the cat studies, a caudate area is exposed by a local decortication and "superfused" by a modification of the cup technique used by Mitchell[7] to demonstrate the cortical release of acetylcholine. Only the *in vitro* studies will be described in this report; the *in vivo* data will be presented in a separate publication.

Methods.—Operative procedure: Sprague-Dawley male rats weighing 200–250 gm were decapitated, brains were immediately removed, and the striatum was dissected with glass manipulators at 4°C. The isolated striata (approximately 50 mg) were incubated separately for 30 min at 37°C in 100 μl of a physiological medium (in mM: NaCl, 126.5; $NaHCO_3$: 27.5; KCl, 2.4; KH_2PO_4, 0.5; $CaCl_2$, 1.1; $MgCl_2$, 0.83; Na_2SO_4, 0.5; glucose, 5.9; adjusted to pH 7.4 with a mixture of O_2 and CO_2, 95 and 5%, respectively) containing 15.6 μCi of L 3–5 ^{3}H-tyrosine (28.2 C/mM, Amersham), and constantly exposed to a flow of the O_2–CO_2 mixture. Immediately after incubation, tissues were washed for 1 min in 10 ml of the physiological oxygenated medium maintained at 37°C. Each striatum was then transferred to a 100-μl organ bath and "superfused" at 37°C with a continuous flow (6 ml/hr) of the oxygenated medium. Serial fractions of effluent medium were collected during 15-min intervals in cooled tubes containing 200 μl of a "protective" solution (ascorbic acid, 1%; EDTA, 1.5%; dopamine, 0.001%) and 8 ml of absolute ethanol. After the "superfusion" period, striata were immediately homogenized in 5 ml of ethanol–water (3:1 v/v). Fractions and homogenates were kept at −17°C until analysis.

Biochemical determinations: The total radioactivity of fractions and homogenate supernatants obtained after centrifugation was assayed in a small aliquot. ^{3}H-dopamine was separated from ^{3}H-tyrosine and ^{3}H-metabolites by ion-exchange chromatography on amberlite CG-50 and by adsorption chromatography on alumina as described previously.[8] The alumina eluates were evaporated to dryness under vacuum, and the radioactivity was measured by liquid scintillation counting. ^{3}H-dopamine and ^{3}H-tyrosine recoveries in alumina column eluates were 60 and 0.01%, respectively, an indication that the ^{3}H-tyrosine overlap in ^{3}H-dopamine determination is negligible. The ^{3}H-tyrosine contamination of the ^{3}H-dopamine never exceeded 5%.

^{3}H-dopamine identification: In some experiments, the identity of ^{3}H-dopamine present in alumina column eluates originating from tissue homogenates or of pooled collected fractions was checked by cochromatography after acetylation of the amine and organic extraction of the acetylated derivative.[9]

Results.—(1) *Synthesis and utilization of ^{3}H-dopamine:* Total radioactivity and ^{3}H-catecholamine contents of isolated striatum were estimated at the end of incubation and at various times during superfusion of the tissues (Fig. 1). The identification (as indicated in *Methods*) of the tissue ^{3}H-catecholamines, one and three hours after the end of the incubation, clearly revealed only the presence of ^{3}H-dopamine. The labeled amine represented 1 per cent of the total radioactivity at the end of the incubation. The ^{3}H-dopamine tissue content increased by at least 65 per cent in the first hour of superfusion, an indication that the amine is still synthesized from its precursor during this period. In the follow-

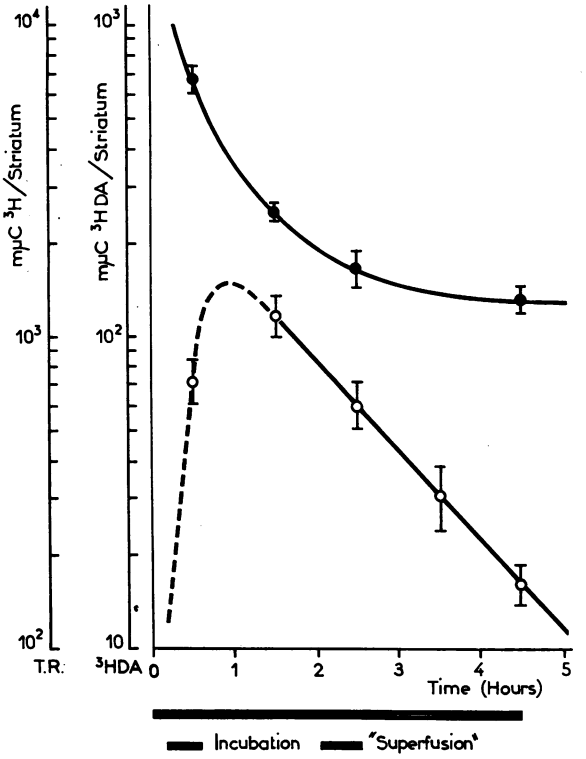

FIG. 1.—Formation and utilization of ^{3}H-dopamine (^{3}HDA) in isolated striata. Isolated striata were incubated for 30 min with ^{3}H-tyrosine and then superfused. ^{3}H-dopamine (*closed circles*) and total radioactivity (*T.R.*) (*open circles*) of tissue contents were estimated at the end of the incubation and at various time intervals of the "superfusion" period. Each value is the mean of four striata ± SE of the mean. The dotted line corresponds to the time interval in which ^{3}H-dopamine is synthesized from ^{3}H-tyrosine.

ing three hours the half-life of the simple exponential disappearance curve of the ^{3}H-amine is about one hour. This is in agreement with the fast turnover of dopamine in this structure as revealed by *in vivo* studies.[10, 11]

(2) *Demonstration of ^{3}H-dopamine spontaneous release:* A spontaneous efflux of ^{3}H-dopamine could be demonstrated in the collected "superfusates." In our experimental conditions, ^{3}H-dopamine released could be accurately estimated during the first two hours following the incubation period. Furthermore, the amine identity was demonstrated by cochromatography of the acetyl derivative. The dopamine identification was performed on pooled fractions obtained from six striata corresponding to the first 15 minutes of "superfusion" and on pooled fractions collected during the following 45 minutes. In both cases, ^{3}H-dopamine could be identified in the alumina eluates of the pooled superfusates and represented total radioactivity of the eluates. ^{3}H-dopamine found in the first fraction represented about 10 to 15 per cent of the tissue ^{3}H-dopamine content. During the first 45 minutes (Fig. 2), the ^{3}H-dopamine level in the successive collected fractions decayed rapidly. In the following 90 minutes, the half-life of this decay was slower, comparable to the tissue ^{3}H-dopamine disappearance in this time interval.

The decline in total radioactivity (mainly ^{3}H-tyrosine, approximately 95%) of the successive collected fractions differs in the initial 45-minute period from that of ^{3}H-dopamine. Similar observations could be made for ^{14}C-urea (15 c/mole; C.E.A., France) and ^{3}H-inulin (75 mc/gm; C.E.N., Belgium) when

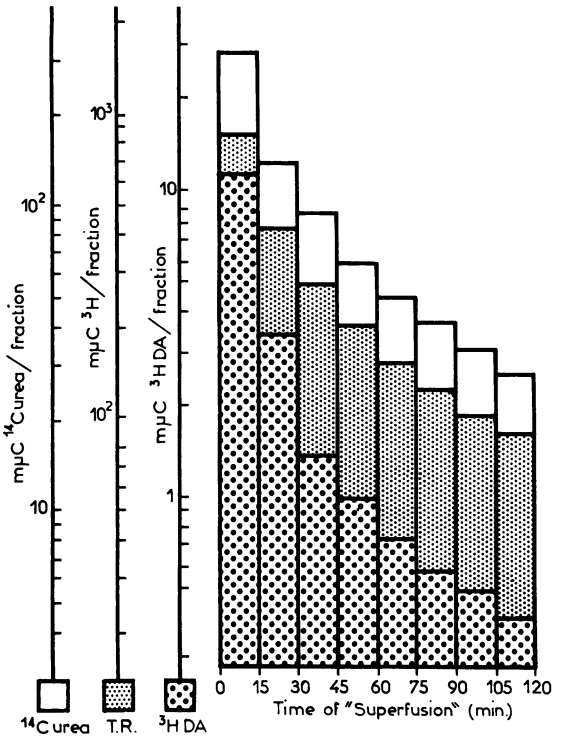

FIG. 2.—Spontaneous release of ^{3}H-dopamine synthesized endogenously from ^{3}H-tyrosine. A typical curve of ^{3}H-dopamine spontaneous release as well as total radioactivity (*T.R.*) output obtained by "superfusing" an isolated striatum previously incubated with ^{3}H-tyrosine. The decay of the ^{14}C output obtained after incubation of isolated striatum with 2.8 μc of ^{14}C-urea under similar conditions is also represented.

isolated striata were previously incubated with these substances which are not, like ^{3}H-dopamine, localized intraneuronally (Fig. 2).

As observed in the various figures, the absolute level of the ^{3}H-dopamine resting release may vary from one experiment to another, but not the shape of the decay curve. This may be due to the relative position of the tissue in the organ bath and the points of arrival and departure of the "superfusing" fluid. In our experimental conditions the ^{3}H-dopamine collected should have originated from the superficial dopamine-containing endings of the striatum.

(3) *Effect of* D-*amphetamine and β-phenylisopropylhydrazine (Catron R) on ^{3}H-dopamine release:* Drugs which affect DA metabolism were tested for their effects on ^{3}H-dopamine extraneuronal appearance. These drugs, dissolved in physiological medium, were continuously introduced for 15 minutes. A marked enhancement of ^{3}H-dopamine content in perfusate could be observed with D-amphetamine concentration as low as 10^{-6} M; higher concentrations (10^{-4} M) produced a similar effect (Fig. 3). When Catron, an inhibitor of monoamine oxidase, was tested (5×10^{-5} M), a marked increase of ^{3}H-dopamine perfusate content was also seen.

(4) *Effect of acetylcholine and 5-hydroxytryptamine on ^{3}H-dopamine release:* The effects of these two substances, normally contained in some striatal neurons, were tested in our preparation. Ach action was always estimated in the presence of eserine (5×10^{-4} M). Eserine alone showed no effect on ^{3}H-dopamine release. When Ach was added at a concentration of 5×10^{-5} M, a marked release

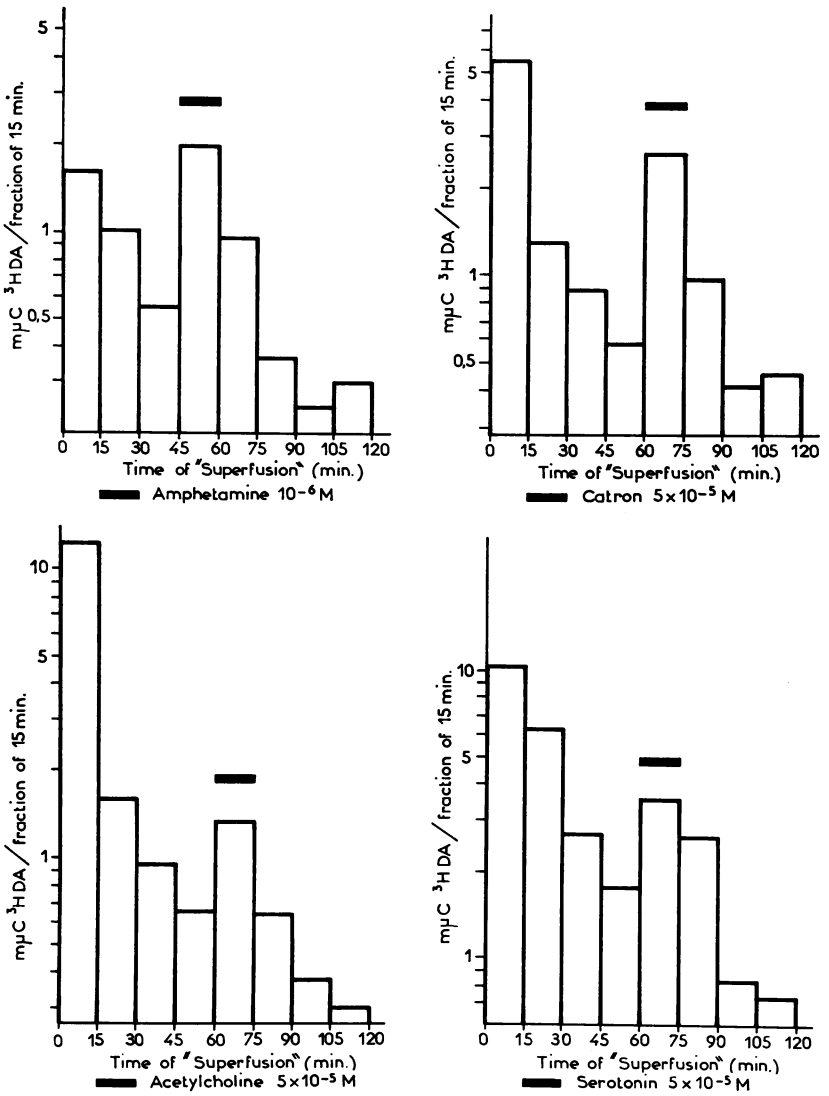

FIG. 3.—Effects of drugs on ³H-dopamine release. Isolated striata previously incubated with ³H-tyrosine were superfused; 45 or 60 min after the onset of the superfusion, drugs were added to the superfusing medium for 15 min (*heavy bars*). ³H-dopamine was estimated in the successively collected superfusate fractions. The figure represents typical effects observed with amphetamine, Catron, acetylcholine, and serotonin.

of ³H-dopamine was seen; no effect could be clearly detected with a 5×10^{-6} *M* Ach solution. An enhanced release of ³H-dopamine was also observed with 5-HT (5×10^{-5} *M*) (Fig. 3). The ³H-dopamine release induced by these drugs appears to be specific; the output of the total tritium, corresponding mainly to ³H-tyrosine, was never affected. Furthermore, it was demonstrated that Ach (5×10^{-5} *M*) did not modify the decay curves of ¹⁴C-urea or ³H-inulin in the collected fractions originating from isolated striata, previously incubated with these substances. In preliminary experiments, in which the effect of ions was

studied, K^+ (20 mM), a depolarizing agent, increased the release of ^{3}H-dopamine.

Discussion.—This *in vitro* study reveals the existence, in the rat striatum, of a resting as well as a drug-inducible efflux of dopamine synthesized intraneuronally from its initial precursor. Both phenomena have also been observed *in vivo* in preliminary studies performed on the cat caudate nucleus. They both seem also to reflect specific mechanisms.

The spontaneous output of ^{3}H-dopamine observed may correspond to a normal resting release of the biogenic amine related to the features of dopamine metabolism in this structure. It should be noted that the curve of ^{3}H-dopamine decay in the initial successive perfusate fractions appears different from those of ^{3}H-tyrosine, ^{14}C-urea, and ^{3}H-inulin. The elimination of the last two compounds represents a diffusion of these substances from the extracellular space into the superfusing medium. Furthermore, under comparable experimental conditions we have observed that the output decay of ^{3}H-5-hydroxytryptamine endogenously synthesized from ^{3}H-tryptophane is slower than that of ^{3}H-dopamine, as well as extraneuronally localized compounds. On the basis of experimental data, the existence of more than one storage pool for catecholamines in central neurons has been proposed.[12, 13] Moreover, it has been shown recently, in peripheral adrenergic neurons, that newly synthesized norepinephrine is preferentially released.[14] Our data suggest that a similar process could operate in central dopaminergic neurons.

The amount of ^{3}H-dopamine collected in the first 15 minutes is about 30 times greater than the ^{3}H-dopamine content of the fraction collected 105 minutes later. However, the tissue ^{3}H-dopamine contents just after the incubation and two hours after the onset of superfusion are almost equal. This suggests that all the ^{3}H-dopamine in the tissue is not equally released; it is consistent with the possibility that newly synthesized ^{3}H-dopamine is preferentially released in the resting state under our experimental conditions.

Acetylcholine and 5-hydroxytryptamine, substances which are normally localized in high concentrations in some striatal neurons or endings and which are believed to act as neurotransmitters, produced a pronounced increase in the output of ^{3}H-dopamine from the tissue. When quantities as low as 10^{-7} moles were administered over a period of 15 minutes, the amount of ^{3}H-dopamine released was 100 per cent greater than the value of the resting release during the previous 15 minutes. An effect could also be generally observed in the fractions collected immediately after 5-hydroxytryptamine or acetylcholine addition. This may be due to the physiological effects of the transmitters on dopamine terminals or to diffusion characteristics of our experimental model. It should be noted that the effect induced by Ach was observed in presence of eserine which by itself showed only a slight or insignificant effect. As previously mentioned, the effect is probably a specific manifestation of the direct action of these substances on dopaminergic endings or on striatal interneurons acting on them. These dopamine terminals belong to the nigro-striatal system of dopamine neurons described on the basis of histochemical, autoradiographic, and lesion studies.[15] The ^{3}H-dopamine is localized in these dopamine terminals. Moreover, Ach had no effect on the output of ^{3}H-tyrosine, probably located

intra- and extraneuronally, nor on ^{14}C-urea and ^{3}H-inulin which were used as indicators of possible inducible physical changes in the extracellular space. Similar effects of Ach on norepinephrine terminals have been described in isolated spleen[16] or heart[17, 18] preparations. The dopamine liberation induced by Ach may explain the effect of some cholinergic drugs, such as oxotremorine, on striatal dopamine metabolism.[19–21] The effects observed with acetycholine, as well as 5-hydroxytryptamine, provide an example of the diversity of the modulating factors acting on the release of dopamine from dopamine neurons.

On the basis of indirect evidence, various authors have suggested that among the various psychotropic drugs which act on the central nervous metabolism of catecholamines, monoamine oxidase inhibitors and amphetamine produce some of their physiological effects by increasing the concentration of catecholamines in the synaptic cleft.[5, 15] The results presented in this report directly demonstrate the rapid extraneuronal increase in dopamine induced by these drugs. This may explain some of their clinical effects, particularly the increased motor activity induced by amphetamine. Both Catron and amphetamine, when administered at concentration 1/10 or 1/50 that of acetylcholine or 5-hydroxytryptamine, produced effects two to three times greater on ^{3}H-dopamine output. In both cases this effect may be due to more than one action of these drugs on dopamine terminals: an increased availability of the amine in a small pool which can be released, a facilitating effect on the release process, and a blockade of the extraneuronal amine inactivation systems, for example, the extraneuronal monoamine oxidase or, more likely, the powerful reuptake process. The intraneuronal increase in catecholamines produced by inhibitors of monoamine oxidase is well known.[5, 15] An effect of amphetamine due to monoamine oxidase inhibition cannot be ruled out; this drug has been shown to produce an increase of brain dopamine levels.[22, 23] Moreover, in *in vitro* studies, brain monoamine oxidase activity can be inhibited by amphetamine (at a concentration of 5×10^{-5} M).[24] Such an action may increase the amount of dopamine available for extraneuronal release. The uptake processes in central norepinephrine and dopamine neurons are different[24, 25] but amphetamine, which inhibits the reserpine-resistant uptake of norepinephine neurons,[5, 24, 25] may also be acting by this mechanism on central dopamine neurons.[25–27] However, these effects are observed *in vivo* only when large doses of amphetamine are administered.[24, 27] It has recently been observed in histochemical studies that amphetamine given in low doses accelerates the disappearance of α-methyl-noradrenaline previously accumulated in DA terminals of rats treated with reserpine and nialamide;[27] this fact was interpreted as a releasing action of the drug on these neurons. This hypothesis is supported and confirmed by our results; it is likely that the marked extraneuronal release of ^{3}H-dopamine produced by low concentration of amphetamine is mainly due to a direct effect of this drug on the process of dopamine release.

These experiments, which reveal the release of dopamine under various pharmacological conditions, give further support to the concept of a neurotransmitter function of dopamine in nigro-striatal dopamine-containing neurons. Moreover, the experimental model employed appears to be a useful tool to investigate

further the interrelationships between neurons acting with different chemical transmitters as well as to study the mechanism of action of some psychotropic drugs.

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