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STUDIES ON THE DISTINCTION BETWEEN UPTAKE INHIBITION AND RELEASE OF [^{3}H]DOPAMINE IN RAT BRAIN TISSUE SLICES

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Abstract—There exists some confusion over the classification of drugs as either uptake inhibitors or releasing agents for biogenic amines. We have evaluated this problem with rat brain tissue slices (neostriatum and cortex), using [^{3}H]dopamine and the following compounds: potassium chloride, tyramine, *d*- and *l*-amphetamine, cocaine, amantadine, desipramine, amitriptyline, nortriptyline and protriptyline. Additional experiments were performed with L-dopa and [^{3}H]serotonin in slices from whole rat brain. Potassium chloride and tyramine, both strong releasing agents, diminished the accumulation of [^{3}H]dopamine during uptake studies. L-Dopa also caused release of [^{3}H]serotonin and an inhibition of [^{3}H]serotonin accumulation. Whenever a releasing action was observed, there was always a diminution in the amount of [^{3}H]amine accumulation and this action (expressed as a per cent effect) was at least equal in magnitude to the per cent released. On the other hand, cocaine was an example of a pure uptake inhibitor; it did not evoke a releasing action at concentrations where a powerful uptake inhibition was seen. From these data, it was possible to conclude that, in the tissue slice system, an experimentally observed release was real (that is, not materially affected by blockade of reuptake), whereas a releasing action evoked an apparent inhibition of uptake equal in magnitude to the releasing action. Before a drug can be designated as an uptake inhibitor, the dose–response curve for inhibition of [^{3}H]amine accumulation should be distinctly to the left of the dose–response curve for release. Our data indicate that, in the neostriatum, all of the drugs studied except cocaine were pure releasing agents and that drugs previously designated as uptake inhibitors were releasing agents. In the cortex, strong inhibition of uptake without significant releasing action was evident for many of the drugs.

In studies of the actions of various drugs in blocking uptake of [^{3}H]biogenic amines or evoking release of stored [^{3}H]biogenic amines, there is a pronounced difficulty in making the experimental distinction between these two separate actions when they occur simultaneously. This problem has been noted by some investigators in studies with brain homogenates, tissue slices or perfusion of brain *in vivo* [1–4]. However, the problem has not been generally appreciated, and guidelines for making the distinction in systems *in vitro* are needed. We have reinvestigated the effects of four commonly used tricyclic anti-depressants as well as amantadine and *d*- and *l*-amphetamine on the accumulation and release of [^{3}H]dopamine in a rat brain tissue slice system. Cocaine and tyramine were used for purposes of comparison, as examples of uptake inhibitors or releasing agents respectively. Our data help to clarify the difficult problem of distinguishing uptake inhibition from a releasing action in this system *in vitro* and indicate that many drugs commonly thought to be uptake inhibitors in the neostriatum are actually releasing agents.

MATERIALS AND METHODS

Incubation medium. A Krebs–Ringer phosphate buffer, pH 7·4, was used throughout. It consisted of 118 mM NaCl, 15·9 mM sodium phosphate, 4·7 mM KCl, 1·8 mM $CaCl_2$, 1·2 mM $MgSO_4$, 1·3 mM EDTA and 5·6 mM glucose. Additionally, 1·7 mM ascorbate was added as an antioxidant [2, 5] and 0·08 mM pargyline (Abbott, Chicago, Ill.) to inhibit monoamine oxidase [2, 5].

Tissue samples. Male Sprague–Dawley rats weighing approximately 150 g were used. The caudate–putamen complex (neostriatum) was dissected in ice-cold saline from the rostral pole to the midportion along the line of the external capsule and the anterior commissure. The caudal neostriatum and the globus pallidus were excluded. A specimen of dorsolateral frontal and parietal cortex overlying the caudate–putamen was also dissected, and the loose meningeal tissue was removed. Three to six rat brains were required to obtain enough neostriatum or cortex for each experiment. Other experiments were run with tissue slices from whole rat brain.

The tissue samples were cut with a razor blade into sections approximately 1 mm thick. They were then cross-chopped at 0·2 mm on a McIlwain–Mickle tissue chopper (Brinkmann Instruments, Westbury, N.Y.). The slices were distributed homogeneously by stirring with a magnetic stirrer for approximately 15 min in 200 vol. (neostriatum or cortex) or 100 vol. (whole brain slices) of ice-cold Ringer's solution.

Uptake of [^{3}*H*]*dopamine.* The uptake of [^{3}H]dopamine ([2-^{3}H]DA, 5·0 Ci/m-mole, New England Nuclear, Boston, Mass., U.S.A.) was measured with modifications of the method of Shaskan and Snyder [5]. The

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[^{3}H]DA used was routinely purified by adsorption onto and elution from aluminum hydroxide [6]. This procedure has been used previously [7]. Quadruplicate aliquots (2 ml) containing 10 mg of slices from rat neostriatum or cerebral cortex were pipetted with an Aupette (Clay-Adams, New York, N.Y.) into 8 ml Krebs–Ringer phosphate medium in 25-ml Erlenmeyer flasks. The tissue slices were equilibrated with shaking for 10 min at 37°. Drugs and the [^{3}H]DA (8×10^5 dis/min, approximately 5×10^{-9} M) were added simultaneously, and the incubation was continued for 15 min at 37°. Samples were then filtered through a Gooch crucible with suction over 2·1-cm Whatman No. 540 filter paper. The tissue slices were rinsed once by suction filtration with 10 ml ice-cold 0·9% saline. The filter paper discs were then transferred to counting vials, and the tissue was extracted for 15 min by incubation with 3 ml absolute ethanol. Fifteen ml Bray's solution was added to each vial, and the vials were assayed for radioactivity by liquid scintillation spectroscopy (counting efficiency, 25 per cent). In some experiments, the ethanol extracts of the tissue slices were concentrated by evaporation under a stream of nitrogen. Thin-layer chromatographic analyses [8] revealed that over 90 per cent of the radioactivity was unchanged [^{3}H]DA in the neostriatum or combined [^{3}H]DA plus [^{3}H]norepinephrine in the cortex. As confirmation, over 90 per cent of the radioactivity in these extracts was bound to aluminum hydroxide [6], indicating very little *O*-methylation of catecholamines.

Release of [^{3}H]dopamine. [^{3}H]DA was taken up into 10 mg of tissue slices as described earlier. The tissue slices were isolated and rinsed by suction filtration. Then, the filter paper discs with adhering tissue slices were suspended in 10 ml fresh Ringer's solution in 50-ml beakers. Incubation with shaking was continued for 15 min at 37° in the presence and absence of various drugs. Each filter paper disc was transferred with a pair of forceps to a Gooch crucible containing a second filter paper disc. The contents were filtered, and the tissue slices were rinsed as before and analyzed for radioactivity.

Uptake or release of [^{3}H]serotonin. Experiments with [^{3}H]serotonin ([1,2-^{3}H]5-HT, 5·0 Ci/m-mole, New England Nuclear) were run as described above except that whole rat brain tissue slices (20 mg rather than 10 mg) were used, and the periods of accumulation and release were 30 min rather than 15 min. The concentration of [^{3}H]5-HT used was approximately 5×10^{-9} M (8×10^5 dis/min).

Kinetic experiments. Both accumulation and release experiments were done as described above except that time of accumulation as well as the time of release were varied.

Analysis of data. Data were calculated as either per cent inhibition of accumulation or per cent release according to the formula:

$$\% \text{ Effect} = \frac{\text{control tissue dis/min} - \text{experimental tissue dis/min}}{\text{control tissue dis/min}} \times 100.$$

The controls in uptake experiments were the tissue samples without drug, corrected for paper blanks. The paper blanks were given by the radioactivity adhering to the filter paper discs for samples without added tissue (400–800 dis/min). Similar blanks were observed for tissue samples taken at zero time or after incubation of tissue slices at 0° for 15 min. The controls in release experiments were the samples after [^{3}H]DA accumulation and subsequent reincubation without added drug, corrected for paper blanks (about 60 dis/min). For examples of data in uptake and release experiments, see legend to Fig. 2. Statistical analyses were carried out using Student's *t*-test.

Drugs used. Drugs used were from the following sources: cocaine hydrochloride (Squibb), desipramine hydrochloride (Ciba Geigy), amitriptyline hydrochloride and protriptyline hydrochloride (Merck, Sharp & Dohme), nortriptyline hydrochloride (Eli Lilly), *d*- and *l*-amphetamine sulfate (Smith, Kline & French), amantadine hydrochloride (E. I. Dupont), L-dopa (Hoffmann–LaRoche) and tyramine hydrochloride (Sigma).

RESULTS

The accumulation of [^{3}H]DA and spontaneous release of previously accumulated [^{3}H]DA were measured at various time intervals (Fig. 1). There was some deviation from linearity of the accumulation of [^{3}H]DA into neostriatal slices by 15 min (the time period of the experiments in Figs. 2 and 3). However, equilibrium had not been reached by 15 min, as there was a further increase in the accumulation of [^{3}H]DA between 15 and 30 min (Fig. 1). The accumulation of [^{3}H]DA in the cortex on the other hand was nearly linear up to 30 min (Fig. 1). There was some spontaneous release (in the absence of added drug) of [^{3}H]DA from both the neostriatum and the cortex (Fig. 1). This spontaneous release was complete by 5 min in the neostriatum; in the cortex there was a further slight increase between 5 and 30 min.

Experiments shown in Figs. 2 and 3 were done with a 15-min accumulation period or a 15-min release period (after a 15-min accumulation). For each drug, there was a dose-dependent inhibition of [^{3}H]DA accumulation by tissue slices from the rat neostriatum (Fig. 2) and cerebral cortex (Fig. 3). The ability of each drug to release previously accumulated [^{3}H]DA (% R) was measured separately and these results have been plotted along with the inhibition of accumulation data (% A) in Figs. 2 and 3.

Inspection of the data reveals the following:

(1) No drug showed greater % R than % A in either the neostriatum or the cortex.

(2) In the neostriatum, the dose-response curves for % A were virtually superimposed on the corresponding curves for % R, for eight of the nine drugs. Cocaine on the other hand showed strong % A without any corresponding % R. Diphenylpyraline (see legend to Fig. 2) similarly showed a strong % A with no significant % R at low concentrations. Tyramine and *d*-amphetamine also showed strong % A, but as noted, this was equivalent to the % R.

(3) In the cortex, the pattern was different. Most drugs caused a strong % A and either no or very much weaker % R. The smallest separation between dose–response curves for % A and % R was shown by tyramine. With regard to potency, tyramine was the strongest releasing agent (50 per cent R at 10^{-6} M); desipra-

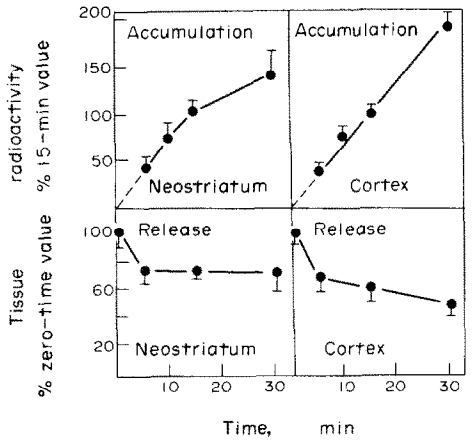

Fig. 1. Accumulation and spontaneous release of [^{3}H]dopamine in slices of rat neostriatum and cortex. All experiments were run in quadruplicate. In accumulation experiments, the tissue slices were incubated at 37° for 5 min, the [^{3}H]dopamine was added and tissue radioactivity measured at 5, 10, 15 and 30 min. Data are expressed as per cent of the 15-min value which was 93,992 ± 9399 dis./min/10 mg of tissue in the neostriatum (mean ± S.D., eight experiments) and 19,434 ± 1166 dis./min/10 mg of tissue in the cortex (three experiments). In separate release experiments, accumulation was done as described above for 15 min. The tissue slices were then filtered and the filter paper disc (with adhering tissue slices) was reincubated in fresh medium at 37° for 5, 15 or 30 min. Tissue radioactivity was then measured. Data are expressed as per cent of the 0 time value which was 101,697 ± 14,234 dis./min/10 mg of tissue in the neostriatum (three experiments) and 15,880 ± 953 dis./min/10 mg of tissue in the cortex (three experiments).

mine was the strongest inhibitor of [^{3}H]DA accumulation (50 per cent A between 10^{-8} M and 10^{-7} M); and amantadine was the weakest in both regards.

Additional experiments were performed with 2×10^{-6} M tyramine in the neostriatum in which the time periods for inhibition of [^{3}H]DA accumulation and release of previously accumulated [^{3}H]DA were 5, 15 and 30 min (Fig. 4). The % A (36 per cent) was slightly greater than the % R (25 per cent) at 5 min. However, the % A and % R were essentially the same at 15 and 30 min.

Potassium chloride, an accepted releasing agent, showed (Fig. 5) a dose-dependent % R equal to the % A in the cortex; in the neostriatum, diminished accumulation of [^{3}H]DA by potassium was also observed, but the % A was somewhat greater than the % R. L-Dopa diminished the accumulation of [^{3}H]5-HT by tissue slices from whole rat brain (Table 1). A releasing action of approximately equal magnitude was also observed (% A = % R).

DISCUSSION

The problem. A number of investigators have noted the difficulties in strict interpretation of uptake or release experiments with [^{3}H]amines [1–3]. However, many investigators have not been aware of the potential pitfall in the interpretation of data. The problem is 2-fold. Consider first the measurement of [^{3}H]amine uptake. During the time that the [^{3}H]amine is taken up, a releasing agent could cause egress of a portion of the accumulated [^{3}H]amine. The resultant diminution in accumulated [^{3}H]amine could be interpreted as decreased "uptake". Therefore, a releasing agent could be misclassified as an uptake blocker. A second difficulty exists in the experimental verification of a presumed releasing action. If tissue containing [^{3}H]amine is incubated in fresh medium at 37°, there is spontaneous efflux of a portion of the [^{3}H]amine [2, 12]. An agent that blocks the reuptake of [^{3}H]amines could, in theory, augment the net loss from the tissue. Since increased net loss is generally construed as constituting a "releasing" action, it is apparent that an uptake blocker could be misclassified as a releasing agent. Thus, there is reason to suspect that uptake blockade and release can become confounded.

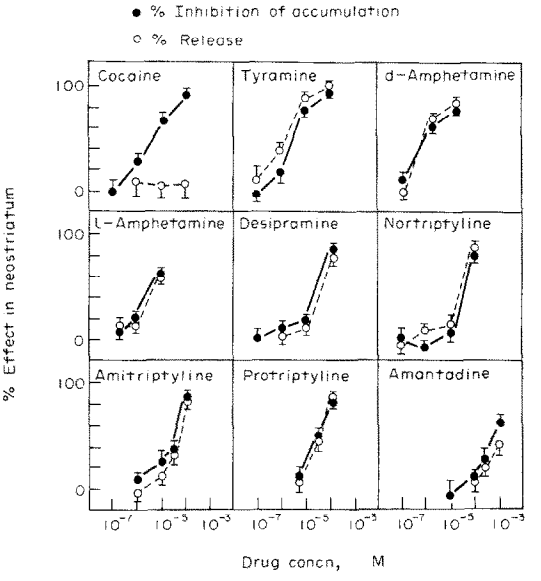

Fig. 2. Effects of various drugs on the accumulation and release of [^{3}H]DA by slices of rat neostriatum. In accumulation experiments, slices were equilibrated at 37° for 10 min and then the drugs and [^{3}H]dopamine were added simultaneously. Tissue radioactivity was measured after 15 min. In release experiments, after [^{3}H]dopamine had accumulated for 15 min, tissue slices were filtered, washed and resuspended in fresh medium. These slices were subsequently incubated with or without drugs for 15 min at 37°. Slices were filtered, rinsed and assayed for radioactivity. Data were calculated by comparison with control specimens as indicated in Materials and Methods. Data are typical experiments run in quadruplicate. Each experiment was repeated several times with similar results. The control samples (no drug) in the accumulation experiments averaged 60,164 dis./min/10 mg of tissue. This represented an accumulation of radioactivity by the tissue approximately 75 times that present in an equal volume of medium. A 50 per cent inhibition of accumulation would be represented by 30,082 dis./min/10 mg of tissue. The spontaneous loss of radioactivity in control samples (no drug) in the release portion of the experiment was a mean of 12,385 dis./min/10 mg of tissue. This diminished tissue radioactivity to an average of 47,779 dis./min/10 mg of tissue for the control samples. A 50 per cent release would be represented by 23,890 dis./min/10 mg of tissue. It should be noted that diphenylpyraline (data not shown), an antihistamine which has been reported to be a catecholamine uptake blocker [9], exhibited 19, 52 and 77 per cent A and only 6, 13 and 34 per cent R at 10^{-6} M, 5×10^{-6} M and $2{\cdot}5 \times 10^{-5}$ M, respectively (mean of quadruplicate samples; this experiment was repeated several times with similar results).

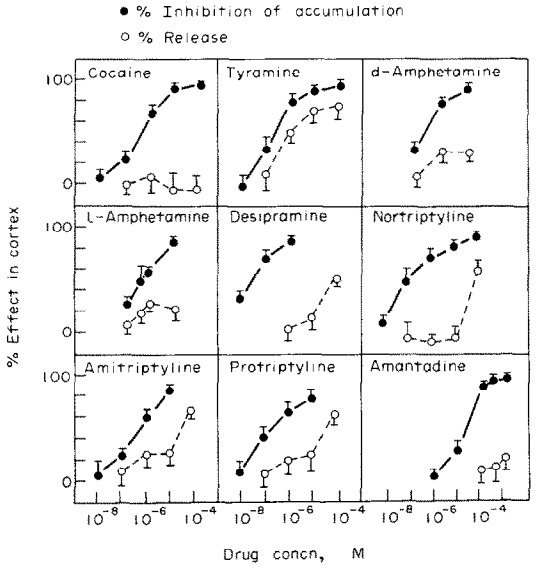

Fig. 3. Effects of various drugs on the accumulation and release of [^{3}H]DA by slices of rat cerebral cortex. Conditions were the same as for the neostriatum (legend to Fig. 2). Data are typical experiments run in quadruplicate. Each experiment was repeated several times with similar results. The control samples (no drug, 0 per cent inhibition of accumulation) for accumulation experiments averaged 16,900 dis./min/10 mg of tissue. This represented an accumulation of radioactivity by the tissue approximately 21 times that present in an equal volume of medium. A 50 per cent inhibition of accumulation would be represented by 8450 dis./min/10 mg of tissue. Spontaneous loss of [^{3}H]DA in release experiments diminished tissue radioactivity to 10,100 dis./min/10 mg of tissue in the control samples (6800 dis./min/10 mg of tissue lost during incubation). A 50 per cent release would be represented by 5050 dis./min/10 mg of tissue. It should be noted that Snyder and Coyle [10] suggested that [^{3}H]DA was transported in the cortex by the norepinephrine transport system. Recent evidence, however, suggests that there may be a dopamine transport system in the cortex (within dopamine-containing nerve terminals) as well as a norepinephrine transport system [11]. The greater number of norepinephrine terminals in the cortex [11] combined with the difference in results between the neostriatum and the cortex (compare Figs. 2 and 3) suggests that the data in the cortex in this study are probably more reflective of the properties of norepinephrine terminals than of dopamine nerve terminals.

The same problems of interpretation exist in whole animal preparations. For example, von Voigtlander and Moore [4] recently observed that perfusion of solutions containing amantadine into the lateral ventricles of cats caused increased efflux of [^{3}H]DA from the caudate nucleus. It was not possible to decide definitively between direct releasing action or blockade of reuptake of spontaneously released [^{3}H]DA as possible mechanisms of action. The authors suggested that blockade of reuptake was the more likely mechanism.

Cocaine as a model uptake inhibitor. Cocaine has gained general acceptance as an inhibitor of the uptake of [^{3}H]amines [13]. In our studies, cocaine eliminated one possible point of confusion in interpreting data. There was in the neostriatum (Fig. 2) and in the cortex (Fig. 3) dose-dependent inhibition of [^{3}H]DA uptake, but no significant release by cocaine even at concentrations as high as 10^{-4} M (the highest tested). Therefore, inhibition of uptake *per se* did not evoke an apparent releasing action. Cocaine was the only compound in the neostriatum in Fig. 2 which showed a dose–response curve for inhibition of accumulation to the left of the dose–response curve for release. However, the situation exemplified by cocaine was not unique. Diphenylpyraline showed similar effects at low concentrations (see legend to Fig. 2). These results mean that, when a releasing action is observed for a drug in the tissue slice system, this action can be accepted as being real, without consideration for any simultaneous action in inhibiting [^{3}H]DA reuptake. This conclusion permits further analysis of the data in the neostriatum as follows.

Potential meaning of the concordance between the per cent inhibition of accumulation (% A) and the per cent release (% R) for drugs acting on neostriatal tissue slices. Eliminating tyramine from consideration, for the moment, it is apparent (Fig. 2) that the other drugs exhibited a broad range of release potencies in the neostriatum. As noted above in the discussion of cocaine, an experimentally observed release does not appear to be confounded by a component due to inhibition of reuptake. Therefore, the apparent releasing effects observed for each of the drugs in Fig. 2 can be accepted as real.

Inspection of Fig. 2 also shows that each of the drugs inhibited the accumulation of [^{3}H]DA. The question arises: How much of this apparent inhibition was due to release of accumulated [^{3}H]amine? The striking thing about the data in Fig. 2 is the concordance between the % A and % R. It hardly seems accidental that this would have occurred for a large group of drugs exhibiting such a wide range of release potencies. One is drawn to consider tentatively that, when a

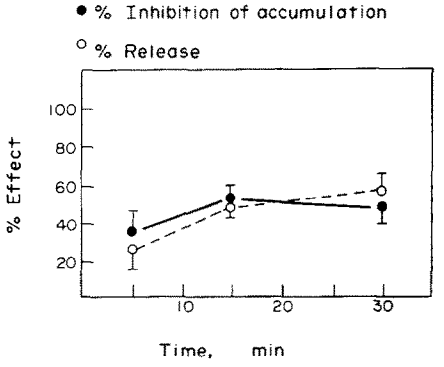

Fig. 4. Effect of 2×10^{-6} M tyramine at several time periods on accumulation and release of [^{3}H]dopamine by slices of rat neostriatum. In accumulation experiments, samples were incubated at 37° for 5 min and then tyramine and [^{3}H]dopamine were added simultaneously. The tissue radioactivity was then measured at 5, 15 and 30 min. The release was done as described in Fig. 2 except that the time of release was either 5, 15 or 30 min after a [^{3}H]dopamine accumulation of the same time period. Data shown are the mean ± S.D. of three experiments each run in quadruplicate. The accumulation of radioactivity by the control samples (no tyramine, 0 per cent inhibition of accumulation) was 29,864 ± 4442, 63,696 ± 12,011 and 83,865 ± 28,075 dis./min/10 mg of tissue at 5, 15 and 30 min respectively. The tissue radioactivity in the control release samples (no tyramine, 0 per cent release) was 23,994 ± 5365, 47,066 ± 7626 and 64,543 ± 9023 dis./min/10 mg of tissue at 5, 15 and 30 min respectively.

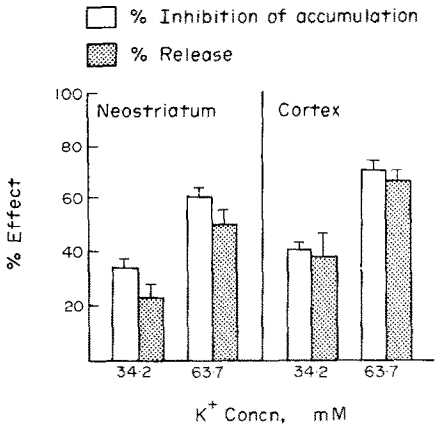

Fig. 5. Effect of KCl on the accumulation and release of [^{3}H]DA in slices of rat neostriatum and cerebral cortex. In these experiments, KCl was substituted for an equimolar amount of NaCl in the medium. Tissue slices were prepared as usual in the Krebs–Ringer buffer. Then, 2 ml containing 10 mg tissue was added to 8 ml high potassium medium at 37° to yield the final concentrations of potassium shown in the figure. Control samples contained the usual 4·7 mM potassium chloride. [^{3}H]DA was then added and tissue radioactivity measured after 15 min. In release experiments, accumulation was done for 15 min in the normal medium. The slices were then filtered, washed and reincubated for 15 min in fresh medium containing various KCl concentrations. They were then assayed for radioactivity. Data shown are the mean ± S.D. for three experiments each run in sextuplicate. The tissue radioactivity present in control accumulation samples (0 per cent inhibition of accumulation) was 60,777 ± 2629 in the neostriatum and 13,591 ± 2908 in the cortex (mean dis./min in 10 mg of tissue ± S.D.). The tissue radioactivity present in release controls (0 per cent release) was 50,528 ± 2150 in the neostriatum and 8635 ± 710 in the cortex (mean dis./min in 10 mg of tissue ± S.D.).

releasing action occurs, it evokes an inhibition of equal magnitude in the accumulation of [^{3}H]DA. In support of this premise, we note that no drug evoked stronger % R than % A. This is in distinction to the converse situation as exemplified by cocaine (Fig. 2), which exhibited a dose–response curve for % A displaced well to the left of the dose–response curve for % R (cf. also other examples in the cortex, Fig. 3). Additionally, it should be noted that in a separate but similar study (H. Orlansky and R. E. Heikkila, submitted for publication) with six other drugs, there was again no example of a drug showing % R greater than % A; three drugs that showed equivalent actions in both systems in the neostriatum were trihexyphenidyl, phenindamine and orphenadrine.

Potassium, tyramine and L-*dopa as model releasing agents.* Potassium and tyramine have gained widespread acceptance as releasing agents for catecholamines [14, 15]. We observed a dose-dependent release of [^{3}H]DA by tyramine (Fig. 2) and by potassium (Fig. 5). Potassium appeared to exert a dual action in the neostriatum as both an uptake blocker and releasing agent (% A somewhat greater than % R). With tyramine in the neostriatum (Fig. 2) and potassium in the cortex (Fig. 5), the effects for % R and % A were virtually identical to one another. Therefore, once again, it appeared that a releasing action evoked an inhibition of equal magnitude in the "uptake" system. This held for both the potassium-evoked release in the cortex (a model for the physiologic release process) as well as for drug-displacement reactions.

Ng *et al.* [12, 16] have shown that L-dopa releases [^{3}H]5-HT from serotonin nerve terminals in brain. This action requires transformation of L-dopa to DA by the decarboxylase enzyme that normally transforms 5-hydroxytryptophan to 5-HT; the newly formed DA appears to "displace" or "release" the [^{3}H]5-HT. In our experiments, the serotonin nerve terminals of the brain were labeled by means of uptake of [^{3}H]5-HT [5, 17]. When L-dopa was added to the tissue slices, there was release of [^{3}H]5-HT (19·2 per cent, Table 1). There was also an identical per cent inhibition of accumulation (20·4 per cent, Table 1). This result lends further credence to the concept that a releasing action evokes an apparent inhibitory effect of equal magnitude in the [^{3}H]amine uptake system.

Interpretation of data for the cortex and neostriatum. Although releasing actions and inhibition of accumulation can, in theory, become confounded, two points have been clarified by the preceding data: (1) experimentally observed release is real (i.e. not affected by concurrent blockade of reuptake) but (2) releasing actions evoke inhibition of equal magnitude in the uptake system. Therefore, before a drug can be designated as an "uptake inhibitor", the dose–response curve for % A should be distinctly to the left of the dose–response curve for % R (% A > % R).

With these considerations, the data in Figs. 2 and 3 can be interpreted as follows. In the neostriatum (Fig. 2) the only compound that actually inhibited uptake of [^{3}H]DA was cocaine (% A > % R). The "inhibition of uptake" reported by others for desipramine, nortriptyline, amitriptyline and protriptyline (four tricyclic anti-

Table 1. Effect of L-dopa ($2·5 \times 10^{-4}$ M) on either the inhibition of [^{3}H]5-HT accumulation or the release of [^{3}H]5-HT from whole rat brain tissue slices*

	Tissue (dis/min ± S.D.)	
	Accumulation	Release
Control	80,088 ± 4476	62,388 ± 8864
+ L-Dopa ($2·5 \times 10^{-4}$ M)	63,736 ± 5640 (20·4%)†	50,468 ± 3496 (19·2%)†

* In accumulation experiments, the [^{3}H]5-HT and L-dopa were added simultaneously and accumulation was measured after 30 min. In release experiments, L-dopa was added to tissue slices which had previously accumulated [^{3}H]5-HT for 30 min. Release was also run for 30 min. Data represent the mean dis/min ± S.D. of [^{3}H]5-HT in the tissue slices (20 mg). This experiment was run in octuplet in a single rat brain; the experiment was repeated three times with similar results. The per cent inhibition of accumulation or per cent release is in parentheses.

† The per cent effects were significant when compared to control ($P < 0·001$ for inhibition of accumulation, $P < 0·005$ for release). These per cent effects were not significantly different from each other ($P > 0·2$).

depressants) or amantadine (an anti-Parkinsonian agent) turns out to be apparent rather than real (% A = % R). The same holds true for *d*- and *l*-amphetamine. All of these compounds have previously been classified as "uptake inhibitors" on the basis of tissue slice or synaptosomal experiments. With our criteria (% A = % R), all drugs except cocaine qualify as releasing agents. Tyramine and *d*- and *l*-amphetamine were the strongest releasing agents studied (% R = 50 per cent or greater at 10^{-5} M).

In the cortex (Fig. 3), a different pattern emerges. All of the nine compounds studied exhibited % A > % R. Several of the drugs (cocaine, desipramine and nortriptyline) were very potent inhibitors of accumulation (e.g. % A = 50 per cent or greater at 10^{-6} M) with no concurrent releasing action. These drugs, therefore, were predominantly uptake blockers in the cortex, although there was some release at high concentrations. Although *d*- and *l*-amphetamine, amitriptyline and protriptyline were predominantly uptake blockers (% A > % R), there may have been some contribution from a release component as well. Amantadine exhibited a relatively weak action as an uptake blocker in the cortex (less than 50 per cent A at 10^{-5} M) but was even weaker as a releasing agent (% A > % R). Tyramine appeared to exhibit a dual action in the cortex, both blocking uptake and causing release. The minimal displacement of % A to the left of % R, however, suggests that most of the observed effect caused by tyramine was due to release.

There was some deviation from linearity of [^{3}H]DA accumulation in the neostriatum by 15 min (Fig. 1). It is probably for this reason that many other investigators [2, 3, 5] have utilized a 5-min time period for accumulation measurements. However, 15 min may be more suitable for release studies. Because a finite time period is probably required for a releasing agent to penetrate sufficiently to reach all of the prelabeled stores, results obtained with short time intervals (e.g. 5 min) may reflect only a portion of the intraneuronal pool of [^{3}H]DA. Longer time intervals (e.g. 15 min) could provide for fuller exposure of the intraneuronal [^{3}H]DA to the releasing action of the drug. It should be noted that this problem is absent during accumulation measurements since both the [^{3}H]DA and the drug are penetrating the tissue simultaneously. This may explain why the % R evoked by 2×10^{-6} M tyramine was slightly less than the % A at 5 min, while the % A and % R were almost identical at 15 and 30 min (Fig. 4). In a separate study (R. E. Heikkila, H. Orlansky and G. Cohen, submitted for publication), we obtained similar results with 2×10^{-6} M *d*-amphetamine in a time course study when the % A and % R were compared.

The problems in interpretation of uptake and release data discussed in the present study suggest a need for the re-evaluation of many compounds thought to be uptake inhibitors in catecholamine or other (e.g. serotonin) uptake systems. In a recent study (H. Orlansky and R. E. Heikkila, submitted for publication), a group of anti-Parkinsonian agents were re-evaluated for uptake inhibition and releasing properties. With the same criteria stated above, some of these agents, previously reported as inhibitors of [^{3}H]DA uptake, were found to qualify as releasing agents in the neostriatum (% A = % R). It seems likely that other drugs now thought to be "uptake inhibitors" (primarily as a result of uptake experiments *in vitro*) may also turn out to be releasing agents.

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