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DOPAMINE UPTAKE INHIBITORS AND RELEASING AGENTS DIFFERENTIATED BY THE USE OF SYNAPTOSOMES AND FIELD-STIMULATED BRAIN SLICES *IN VITRO*

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Abstract—Rat brain striatal synaptosomes or slices *in vitro* have been used to test the DA uptake inhibitory or releasing properties of certain drugs and to differentiate between these two processes.

DA uptake into synaptosomes rapidly reaches saturation and the choice of incubation time is important for measuring inhibition. Thus nomifensine is apparently less active as an inhibitor when the incubation time is extended beyond the linear phase of uptake. As overall DA uptake reaches a plateau an extensive exchange of DA still occurs, presumably through a process of spontaneous release and reuptake. This phenomenon may provide an explanation for the apparent releasing properties of uptake inhibitors, the action of which would tend to favour the spontaneous release.

Both nomifensine and amphetamine in high concentrations cause an increase in the amount of radioactivity in the medium when they are incubated with synaptosomes preloaded with [^{3}H]DA. Their combined effects, however, are not additive, showing that different mechanisms must be involved. The difference is emphasised when the drugs are introduced under superfusion conditions where reuptake of DA is minimised. Amphetamine shows a very strong releasing effect, whereas nomifensine is inactive. Benztropine and methylphenidate both behave like nomifensine in these conditions.

Similar results are found using preloaded, electrically-stimulated striatal slices, but in this case methylphenidate, as well as amphetamine, increases the overflow of radioactivity. With respect to newly-accumulated [^{3}H]DA, nomifensine and benztropine can thus be considered as purely uptake inhibitors. The different releasing properties of methylphenidate and amphetamine may provide an explanation for their pharmacological differences.

While many compounds have been described as inhibitors of the neuronal uptake of serotonin (5-HT) and noradrenaline (NA), relatively few compounds are known which are strong inhibitors of dopamine (DA) uptake [1–3]. The *in vitro* methods currently used for measuring uptake inhibition, however, do not readily distinguish inhibitors from drugs causing release of newly-captured transmitter and for certain drugs, such as amphetamine in particular, there has been much discussion concerning the relative importance of each process [4–6]. In a recent report, Baumann and Maitre [7] have postulated that, for a whole series of inhibitors, apparent inhibition of [^{3}H]DA uptake into synaptosomes can be correlated with depletion of total DA content, thus implying that DA release is the predominant, if not the only, process involved. This effect was not observed in the case of NA. Using several different techniques *in vitro* we have demonstrated that it is possible to distinguish drugs acting purely as DA uptake inhibitors from those which mainly enhance release. In this respect, the antidepressant nomifensine is compared with the two psychostimulants, amphetamine and methylphenidate and with the antiparkinson agent, benztropine.

MATERIALS AND METHODS

General

Immature female (18–21 days) albino rats were used in all experiments unless otherwise specified. All reagents used were of analytical grade. DL-[Methylene-^{14}C]noradrenaline DL-bitartrate (55 mCi/m-mole), L-[7-^{3}H]noradrenaline hydrochloride (10.9 Ci/m-mole), [ring-G-^{3}H]dopamine hydrochloride (500 mCi/m-mole) and [ethylamine-1,2-^{3}H]dopamine hydrochloride (2.3 Ci/m-mole) were supplied by the Radiochemical Centre (Amersham, Bucks., England). Radioactivity measurements were made by liquid scintillation.

Monoamine uptake in vitro

Crude synaptosomal fractions from rat brain were obtained according to the method of Whittaker [8]. Our methods for determining monoamine uptake were similar to those of Snyder and Coyle [9] and have been described in detail elsewhere [10]. Synaptosomal uptake of [^{14}C]NA was measured using Krebs–Henseleit bicarbonate buffer (pH 7.4), containing 11 mM glucose, while phosphate buffer was used for studying [^{3}H]-DA uptake in synaptosomes from the corpus striatum [11]. Aliquots (2.5 ml) were taken from the synaptosome suspensions, and the samples were incubated with labelled monoamine (1×10^{-7} M final concentration) at 37° in a shaking water bath in the presence or absence of drugs. Incubation time was 10 min with synaptosomes from hypothalamus and 3 min (or other periods of time as indicated in Table legends) with striatal synaptosomes. The reaction was terminated by cooling the tubes in ice. To determine nonspecific adsorption, control samples were incubated at 0° under identical conditions.

The amount of accumulated monoamine was evaluated by a membrane filtration technique [12], using a Millipore sampling manifold (Millipore GmbH. Neu-Isenburg) with cellulose nitrate filters, 25 mm in diameter and 0.6 μm pore size (Sartorius GmbH., Göttingen). After harvesting the synaptosomes under mild vacuum, the filters were washed once with the respective buffer (5 ml) and dissolved directly in a dioxane based scintillation fluid (10 ml) containing PPO (2,5-diphenyloxazole, 0.6%, w/v) and water (1.2%, v/v). Radioactivity of the samples was determined and the amount of monoamine accumulated by the synaptosomes was expressed as the percentage of the radioactivity added to the incubation mixture.

IC_{50} values were evaluated as the concentration of drug inhibiting the specific uptake of either [^{14}C]NA or [^{3}H]DA by 50 per cent. For each drug, at least 3–4 different concentrations were used in triplicate.

In experiments involving a pre-incubation with unlabelled NA or DA the amine at a concentration of 10^{-7} M was included in the incubation buffer. [^{3}H]DA or [^{3}H]NA at a concentration of 10^{-8} M was added either simultaneously with the respective unlabelled amine or during the incubation as indicated.

Release of [^{3}H]DA from preloaded synaptosomes

(*a*) *By resuspension.* Rat striata were homogenised in 0.32 M sucrose solution (1:20, w/v). The homogenate (4 ml) was centrifuged at 1000 *g* for 10 min and the supernatant diluted 10-fold with Krebs–Henseleit bicarbonate buffer containing 11 mM glucose, 0.025 mg/ml EDTA, 0.2 mg/ml ascorbic acid and 10^{-4} M iproniazide. The suspension was incubated for 20 min at 37° in the presence of [^{3}H]DA (10^{-6} M). After cooling in ice-water it was centrifuged at 16,000 *g* for 10 min and the resulting pellet was rinsed superficially and resuspended in buffer (60 ml). 2.5 ml fractions were pre-incubated at 37° for 2 min before drug addition. Incubations were continued for various periods of time and stopped by cooling in ice. Subsequent filtration and radioactivity measurement were as described for monoamine uptake *in vitro*.

(*b*) *By superfusion.* The preparation was essentially as described above. Striata were homogenised in 12 vol. 0.32 M sucrose. The supernatant (~6 ml) from the debris centrifugation was diluted to 30 ml with a modified Krebs–Tris buffer (pH 7.4) containing 128 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 15 mM Tris, and in addition 5 mM D-glucose, 1.25×10^{-5} M nialamide and 0.2 mg/ml ascorbic acid. The suspension was incubated for 10 min at 37° in the presence of [^{3}H]dopamine (10^{-7} M) and aliquots (4 × 6 ml) were subsequently filtered on Whatman GF/C glass-fibre discs held in thermostated (37°) glass jackets as described by Raiteri *et al.* [13]. The filters were washed first under low vacuum with (3 × 5 ml) buffer at 37°, then under superfusion conditions at a rate of ~1 ml/min with oxygenated buffer and with a head of 1 ml maintained over the filter. One minute fractions were collected and after 5 min buffer containing 2.7 mM $CaCl_2$ with or without drug was introduced. Superfusion was continued for a further 15 min and an aliquot (500 μl) was taken from each fraction for radioactivity measurement. The radioactivity in each fraction was expressed as the percentage of the total radioactivity released plus that remaining on the filter, which was measured after digestion overnight in scintillation fluid containing 30% Triton X-100.

Electrical stimulation of brain slices

The method used for studying drug effects on the release of dopamine from electrically stimulated brain slices is based on that of Farnebo and Hamberger [14] and has been described in detail elsewhere [15]. Slices from rat brain striatum were incubated for 30 min at 37° in a Krebs bicarbonate buffer (pH 7.4) with [^{3}H]DA (1×10^{-7} M, 2.7 Ci/m-mole). The slices were rinsed and superfused at a rate of 0.5 ml/min with buffer in the presence or absence of drug. After 30 min the slices were stimulated for 2 min by rectangular biphasic pulses of 2 msec with 10 Hz and 14 mA. Superfusion was continued for 18 min and 5 min fractions were collected for radioactivity measurement. Radioactivity remaining in the slice was determined and the stimulation-induced overflow of radioactivity was calculated by subtracting the estimated spontaneous efflux from total efflux during the same period. It is expressed as a percentage of the radioactivity content of the slice at the onset of stimulation.

RESULTS

Catecholamine uptake in vitro

Table 1 shows the *in vitro* activities of some DA uptake inhibitors with respect to both DA and NA in crude synaptosomal preparations from rat corpus striatum or hypothalamus respectively. The tricyclic antidepressant desipramine is also included as the reference compound for NA uptake inhibition. As previously reported [2, 10] nomifensine is an effective inhibitor of uptake of both NA and DA. While methylphenidate has a similar order of activity towards DA it is 10 times less active than nomifensine in inhibiting NA uptake.

As shown in Fig. 1, DA uptake in striatal synaptosomes occurs very rapidly, an equilibrium being reached after 6 min. Uptake cannot be assumed to be linear after the first 3 min of incubation. As the incubation time is extended the apparent inhibition by nomifensine decreases. The IC_{50} values for 3 min and 10 min incubations are, respectively, 0.085 μM and 0.25 μM.

Table 1. Inhibition of [^{14}C]NA and [^{3}H]DA uptake *in vitro*

	IC_{50} (μM)	
	[^{14}C]NA	[^{3}H]DA
Nomifensine	0.032	0.085
D-Amphetamine	0.10	0.64
Methylphenidate	0.36	0.25
Benztropine	0.80	0.31
Desipramine	0.025	20.0

The synaptosomal preparation from rat hypothalamus or corpus striatum was incubated with [^{14}C]NA or [^{3}H]DA respectively, both at 10^{-7} M and in the presence or absence of inhibitor. Incubation was for 10 min for NA and 3 min for DA. For each inhibitor 3–4 concentrations were used in triplicate. IC_{50} values were determined from logarithmic probability plots of inhibitor concentration against percentage inhibition of [^{14}C]NA or [^{3}H]DA uptake after subtraction of 'non-specific' incorporation, estimated by an identical incubation of each radioactive amine at 0°.

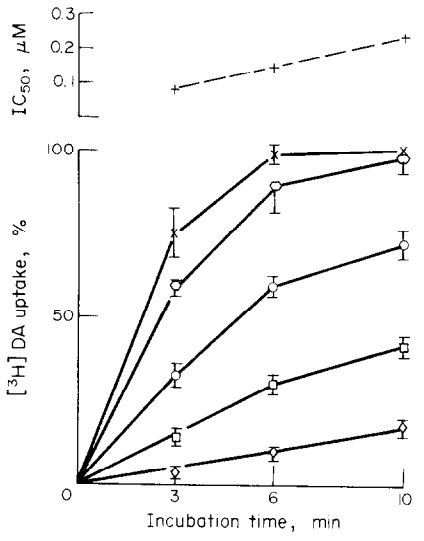

Fig. 1. Time dependence of [^{3}H]DA uptake inhibition in striatal synaptosomes. The synaptosomal preparation from rat corpus striatum (final dilution 1:300, w/v) was incubated with different concentrations of nomifensine for 3, 6 or 10 min. Incorporation of radioactivity into the synaptosomes is shown as a percentage of maximum net incorporation which was 9–14 per cent of the total radioactivity. Control, ×; nomifensine 3×10^{-8} M, ⬭; 1×10^{-7} M, ○; 3×10^{-7} M, □; 1×10^{-6} M, ◇. Each value represents the mean (±S.D.) of 3–5 experiments done in duplicate. In the upper part the IC_{50} evaluated by plotting the log of inhibitor concentration against the uptake inhibition as percentage of the respective control uptake is shown as a function of incubation time.

Uptake of labelled catecholamines after pre-incubation of synaptosomes with the unlabelled compounds

The time course of [^{3}H]DA uptake into a crude synaptosomal preparation is shown in Table 2. When the synaptosomes are incubated with a tracer concentration of labelled amine (10^{-8} M), simultaneously with unlabelled amine (10^{-7} M), incorporation of radioactivity reaches a plateau after 10 min. If, however, the synaptosomes are incubated for 2 min with the tracer after various periods of pre-incubation with the unlabelled amine, incorporation of radioactivity reaches a constant level of about 35 per cent of maximum incorporation as the overall uptake ceases. In other words, an equilibrium between spontaneous release and reuptake of DA is established, and results in a rapid exchange of free DA with that within the synaptosomes.

Similar experiments have been carried out for NA and in this case overall uptake is slower, reaching a plateau after 30 min (Table 3). Radioactive NA can also be incorporated when overall uptake has virtually ceased, but the exchange is less extensive than for DA.

Release of [^{3}H]DA from preloaded synaptosomes

(*a*) *By resuspension.* When synaptosomes, preloaded with [^{3}H]DA, are resuspended and incubated at 37°, there is a spontaneous release of radioactivity into the medium (Fig. 2). Radioactivity in the medium is slightly increased by nomifensine (10^{-5} M)* and strongly increased by D-amphetamine (2×10^{-5} M). If,

* No further increase is observed with higher concentrations of nomifensine.

Table 2. Uptake of [^{3}H]DA after preincubation with unlabelled DA

Total incubation time (min)	Maximum uptake of [^{3}H]DA (%) Tracer added at 0 min	Tracer added 2 min before end of incubation
2	50 ± 4	
4	79 ± 6	50 ± 5
6	91 ± 4	36 ± 3
8	96 ± 7	34 ± 7
10	100	35 ± 6

The synaptosomal preparation from rat corpus striatum was incubated in the presence of unlabelled DA (10^{-7} M) for various periods of time up to 10 min as indicated. [^{3}H]DA (10^{-8} M) was either added simultaneously with unlabelled DA or 2 min before the end of incubation. Incorporation of radioactivity into the synaptosomes is expressed as a percentage (±S.D.) of maximum incorporation which represented 8–10 per cent of the total radioactivity.

Table 3. Uptake of [^{3}H]NA after preincubation with unlabelled NA

Total incubation time (min)	Maximum uptake of [^{3}H]NA (%) Tracer added at 0 min	Tracer added 2 min before end of incubation
5	37 ± 6	21 ± 9
10	56 ± 6	14 ± 5
20	81 ± 13	12 ± 6
30	100	12 ± 6

The conditions were exactly as described for DA (Table 2) except that the synaptosome preparation was from rat hypothalamus and incubations were continued for up to 30 min.

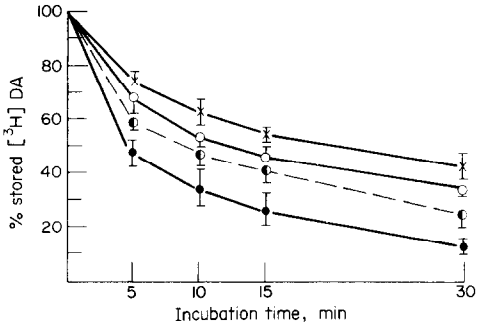

Fig. 2. Release of [^{3}H]DA from preloaded striatal synaptosomes. Synaptosome suspensions were preloaded by incubation with [^{3}H]DA (10^{-6} M) for 20 min at 37°. After cooling in ice, the synaptosomes were separated by centrifugation, and the pellet was rinsed and resuspended in fresh buffer. Fractions of 2.5 ml were preincubated for 2 min at 37° in a shaking water bath, then the drugs were added (zero time), and incubation was performed for different periods as indicated. Control, ×; nomifensine 1×10^{-5} M, ○; amphetamine 2×10^{-5} M, ●; nomifensine 1×10^{-5} M + amphetamine 2×10^{-5} M, ◑. The percentage of radioactivity remaining in the particulate fraction was evaluated from 5–6 independent determinations in duplicate after various incubation times. The results are given as mean values ±S.D.

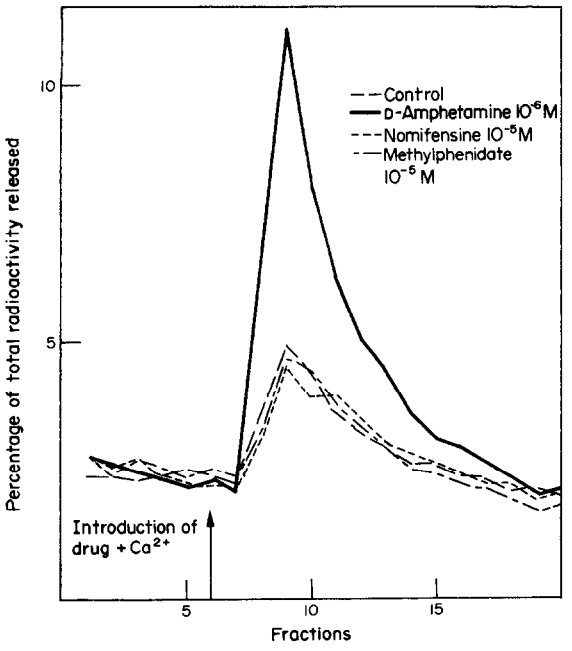

Fig. 3. Release of [^{3}H]DA from striatal synaptosomes using a superfusion technique. Synaptosomes from rat corpus striatum were preloaded with [^{3}H]DA, filtered on Whatman GF/C glass fibre discs, washed and then superfused with buffer at 37° at a rate of 1 ml/min. Drugs were introduced as indicated simultaneously with 2.7 mM $CaCl_2$. Superfusion was continued for a further 15 min and 1 min fractions were collected for radioactivity measurement. The radioactivity in each fraction is expressed as the percentage of the total radioactivity released plus that remaining on the filter.

however, nomifensine and amphetamine are added together at the same concentrations, their effects are not additive and the resulting curve is intermediate between those for each of the drugs alone.

(*b*) *By superfusion.* The superfusion technique described by Raiteri *et al.* [6] is particularly useful for detecting release of transmitter substances from preloaded synaptosomes because under superfusion conditions reuptake of released transmitter is minimal. The technique has been slightly modified to give a more sensitive response. Preloading with [^{3}H]DA and washings are carried out with Ca^{2+}-free buffers, and Ca^{2+} at a concentration of 2.7 mM is only introduced into the superfusion medium at the same time as the test drug. In this way, more consistent results are obtained and the effect of releasing agents is more pronounced.

Table 4. DA release by superfusion: Induced net overflow of radioactivity

		Drug induced release (%)
D-Amphetamine	10^{-6} M	19.0 ± 1.5
Nomifensine	10^{-5} M	−3.5 ± 0.8
	10^{-6} M	−1.9 ± 1.5
Methylphenidate	10^{-5} M	−1.0 ± 0.7
	10^{-6} M	−1.7 ± 0.4
Benztropine	10^{-6} M	−3.0 ± 0.3
K^+	56 mM	18.9 + 1.2

Radioactivity released over 9 min after drug or K^+ introduction (fractions 7–16 inclusive in Fig. 3) is expressed as a percentage of total radioactivity (all fractions plus filter). Background release in controls (equal to 32.9 ± 0.9 per cent of total, $n = 9$) is subtracted. Values represent the average (±S.E.M.) of at least 3 determinations.

As shown in Fig. 3, D-amphetamine at a concentration of 10^{-6} M causes a strong release of radioactivity, while both nomifensine and methylphenidate at a concentration of 10^{-5} M have no effect compared with the control where Ca^{2+} only is introduced. It is of note that Ca^{2+} by itself is sufficient to cause some release.

The results of a number of such experiments are summarised in Table 4 where the release of radioactivity above the controls is expressed as a percentage of the total radioactivity in the fractions plus the filter. Like nomifensine and methylphenidate, the DA uptake inhibitor benztropine (10^{-6} M) does not cause release above the control value; a similar result has been reported by Raiteri *et al.* [16]. Increasing K^+ concentration to 56 mM with concomitant reduction in Na^+ concentration strongly stimulates release and it has been verified that this effect is Ca^{2+}-dependent.

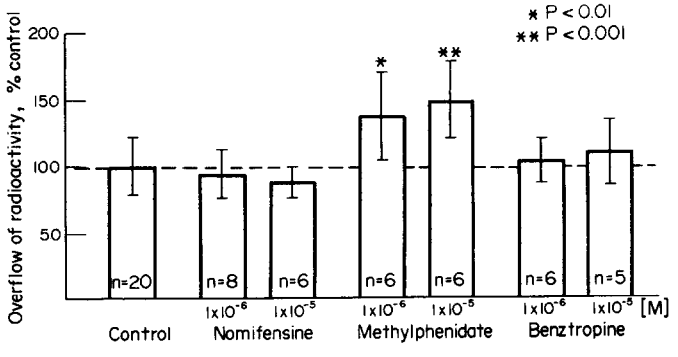

Fig. 4. Drug-induced changes in the overflow of [^{3}H]DA from electrically stimulated slices of rat corpus striatum. Striatal slices preloaded with [^{3}H]DA were superfused for 30 min with buffer at 37° at a rate of 0.5 ml/min and in the presence or absence of drug. Stimulation was applied for 2 min with an electrical field characterized by square wave biphasic pulses of 2 msec, 14 mA and 10 Hz. Superfusion was continued for 18 min and 5 min fractions were collected for radioactivity measurement. Stimulation induced efflux of radioactivity is expressed as a percentage of the radioactivity content of the slice at the onset of stimulation and the values represent the means (±S.D.) for *n* slices. Statistical significance was determined using Student's *t* test.

Overflow of [^{3}H]DA from field stimulated slices of rat corpus striatum

Another method for studying drug effects on the release of newly-accumulated transmitter is by the electrical stimulation of preloaded brain slices superfused by the drug solution.

When rat brain striatal slices preloaded with [^{3}H]DA are superfused with solutions of nomifensine or benztropine at 10^{-5} or 10^{-6} M (Fig. 4) there is no increase of radioactivity released into the medium compared with that from control slices without drug. Methylphenidate, on the other hand, significantly stimulates radioactivity release at the same concentrations, although its effect is less than that previously reported for amphetamine [15].

DISCUSSION

The different techniques employed for measuring the release of newly-accumulated [^{3}H]DA in rat brain striatal synaptosomes or slices allow a clear discrimination between uptake blockers and releasing agents.

These results demonstrate that the equilibrium which is established between spontaneous release of DA and its recapture (for discussion see Schacht *et al.* [15]), is characterised by a rapid exchange of the transmitter, in which case introduction of an uptake inhibitor could lead to a depletion of DA content by favouring its spontaneous release.

This hypothesis provides an explanation for the observation of Baumann and Maitre [7] that the degree of uptake inhibition can be correlated with a decrease in endogenous DA content. Their experimental conditions particularly lend themselves to this situation because uptake inhibition was measured with an incubation time of 10 min, that is, when overall uptake is no longer linear, and an equilibrium state has already been reached. The importance of using a short incubation time, corresponding to the initial linear phase of DA uptake, is emphasized by the results shown in Fig. 1 where the apparent IC_{50} for nomifensine rises from 0.085 to 0.24 μM as the incubation time is extended from 3 to 10 min. As equilibrium is approached the exchange of DA is extensive, as shown by the addition of a tracer amount of labelled amine just before the end of an incubation with unlabelled amine. In the case of NA, the overall uptake is slower (30 min to reach a plateau) and at equilibrium the extent of exchange over the same period is less than with DA. This difference may explain why no similar decrease of NA content is observed after incubation of synaptosomes with NA uptake inhibitors.

Although nomifensine at a concentration (10^{-5} M) much higher than that required to inhibit DA uptake by 50 per cent causes some increase in radioactivity in the medium when preloaded synaptosomes are incubated in the presence of the drug (Fig. 2), this effect can be explained simply as being due to inhibition of reuptake of spontaneously released DA. Amphetamine, which is generally accepted to be predominantly a releaser rather than an uptake inhibitor [5, 6], not only causes more release of DA from preloaded, resuspended synaptosomes than does nomifensine, but its effect is diminished rather than reinforced when the two drugs are incubated simultaneously, thus showing that in these conditions their effects are opposite. Similar antagonism of amphetamine induced DA release by the uptake inhibitor benztropine has been reported by Raiteri *et al.* [16] using a superfusion system. These antagonistic effects can only be explained by different modes of action of amphetamine on one hand and of nomifensine and benztropine on the other hand. With the superfusion technique, where reuptake of released DA is minimal, the difference between amphetamine and nomifensine is even more marked. Amphetamine at a concentration of 10^{-6} M strongly stimulates release to the same extent as depolarisation by 56 mM K^+. In contrast, neither nomifensine, benztropine, nor methylphenidate increase release above the control value at concentrations of 10^{-5} or 10^{-6} M.

Nomifensine and benztropine, moreover, do not increase electrically stimulated DA release from striatal slices either, and thus in this respect they differ from both amphetamine [15] and methylphenidate.

Incidentally, the difference seen here between amphetamine, which enhances both spontaneous and stimulated release, and methylphenidate, which enhances only stimulated release, may explain their different behavioural effects in animals pretreated with either reserpine or inhibitors of catecholamine biosynthesis (for references see [17]).

These results thus demonstrate that nomifensine, as well as benztropine, can be considered as DA uptake inhibitors and not releasers, at least as far as newly-accumulated [^{3}H]DA is concerned. Of the compounds chosen by Baumann and Maitre [7] as DA uptake inhibitors and which were shown to deplete total synaptosomal DA content, only nomifensine, benztropine and amphetamine are active at concentrations where non-specific interactions with membranes may be considered unimportant, so emphasis is placed on these three drugs. While amphetamine, having primarily a releasing effect, might be expected to cause depletion of synaptosomal DA, this is not the case for nomifensine and benztropine with appropriate experimental conditions *in vitro*, as demonstrated here. A less likely explanation for the DA depletion observed by Baumann and Maitre is that the uptake inhibitors may also deplete vesicular stores of DA rather than newly accumulated [^{3}H]DA which represents only a small proportion of the total (4 per cent according to the same authors) and which may behave differently. It is hard to reconcile this possibility, however, with the results of White [18] which show that newly captured radioactive catecholamines very rapidly label vesicular stores.

Both nomifensine and benztropine have been found to be active in inhibiting dopamine uptake *in vitro* following i.p. or oral administration *in vivo* [19, 20, M. Leven and P. Hunt, unpublished results]. That Baumann and Maitre [7] found only slight activity may be explained by their choice of experimental conditions as discussed above. In the case of nomifensine there is indirect evidence for uptake inhibition *in vivo* without DA depletion. Nomifensine at a dose of 10 mg/kg blocks the depletion of DA induced by 6-hydroxydopamine [21] and the latter apparently exerts its effect only after being incorporated into catecholaminergic neurons by the uptake process [22]. At the same dose nomifensine does not alter DA levels.

The apparent differences between the antidepressant nomifensine and the psychostimulants amphetamine and methylphenidate should also be emphasized, since

similarities between nomifensine and methylphenidate have recently been suggested [17, 23], both drugs causing stereotyped behaviour and increased formation of DA metabolites (HVA and DOPAC), following their administration to rats in relatively high doses (10, 30 or 60 mg/kg, s.c.). As previously mentioned, methylphenidate, like amphetamine, significantly increases stimulated release of DA from brain slices, while nomifensine has no effect. In the case of nomifensine therefore, increased brain concentrations of HVA and DOPAC after high doses may reflect an increase in DA turnover rather than enhanced release. Such an effect could be mediated by cAMP since nomifensine, though not stimulating DA-sensitive adenylate cyclase *in vitro* [U. Schacht, unpublished results], increases striatal cAMP concentrations dose-dependently [24] and it has been shown [25] that, in synaptosomes, dibutyryl cAMP can stimulate DA turnover independently of DA release.

Another important difference between nomifensine and methylphenidate is with respect to the noradrenergic system. As an inhibitor of NA uptake, nomifensine is 10 times more active than methylphenidate both *in vitro* and after *in vivo* administration [U. Schacht and P. Hunt, unpublished results] having an activity similar to that of desipramine.

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