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Effect of long term amineptine treatment on pre- and postsynaptic mechanisms in rat brain

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1 The effect of amineptine and its two metabolites on monoamine uptake, release and receptor binding was studied *in vitro*.

2 Amineptine and its two metabolities did not displace labelled ligands for known neurotransmitters and drug receptor sites.

3 Amineptine and its two metabolities did not influence [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) uptake or release by rat brain synaptosomes. Amineptine inhibited [^{3}H]-dopamine and [^{3}H]-noradrenaline ([^{3}H]-NA) accumulation, with IC_{50} values of 1.4 and 10 μM, respectively. The effect was retained, though with lower efficacy, by the two metabolites.

4 Amineptine released [^{3}H]-dopamine from preloaded synaptosomes. Metabolite 1 had no effect on catecholamine release, and metabolite 2 was about half as active as the parent compound on [^{3}H]-dopamine release.

5 The releasing effect of amineptine on [^{3}H]-dopamine was potentiated by reserpine pretreatment, suggesting that the drug acts on the cytoplasmic neurotransmitter pool.

6 Chronic treatment with amineptine (20 mg kg^{-1}, twice daily for 15 days followed by a 3 days drug withdrawal period) resulted in a decrease of [^{3}H]-spiperone binding sites in striatum, and of [^{3}H]-dihyroalprenolol and [^{3}H]-clonidine in cortex.

7 Chronic treatment with amineptine reduced basal [^{3}H]-dopamine accumulation in striatal synaptosomes, without affecting [^{3}H]-NA or [^{3}H]-5-HT accumulation.

8 The adaptive changes in the pre- and postsynaptic dopamine mechanisms observed after long term treatment with amineptine are consistent with the drug acting as an indirect dopamine agonist.

9 The down regulation of β- and α_2-noradrenoceptors observed after long term amineptine treatment may play a role in the antidepressant activity of the drug.

Introduction

Amineptine, an antidepressant drug (Samanin *et al.*, 1977; Roster, 1979) effective in several screening tests for antidepressant activity (Poignant, 1979) and particularly on the Porsolt immobility test (Borsini *et al.*, 1981), is characterized by a selective effect on the dopaminergic system (Samanin *et al.*, 1977; Dankova *et al.*, 1977). Like other dopamine receptor agonists, amineptine increases locomotor activity and induces stereotyped movements and hyperthermia in rats (Samanin *et al.*, 1977), it appears to inhibit dopamine uptake (Algeri *et al.*, 1978); it raises rat striatal homovanillic acid (HVA) levels (Samanin *et al.*, 1977; Dankova *et al.*, 1977) and reduces dopamine turnover (Algeri *et al.*, 1978), without significantly affecting 3,4-dihydroxyphenylacetic acid (DOPAC).

Amineptine differs from other tricyclic agents in its side chain, which is 7-aminoheptanoic acid (Figure 1). Kinetically amineptine is rapidly adsorbed and metabolized, to form two major metabolites (Figure 1) resulting from β-oxidation of the lateral chain, giving rise therefore to a tricyclic agent with 5-aminoeptanoic acid (metabolite 1) or 3-aminopentanoic acid (metabolite 2) (Sbarra *et al.*, 1979; 1981).

In this study an attempt was made to characterize further the mechanism of action by investigating the effects of the drug and its two metabolites on uptake, release and binding of ^{3}H-monoamines from synaptosomal preparations *in vitro*. Moreover, since the effect of antidepressants become evident only after repeated drug treatment, we have studied ^{3}H-monoamine uptake, release and binding *ex vivo*, after chronic amineptine administration.

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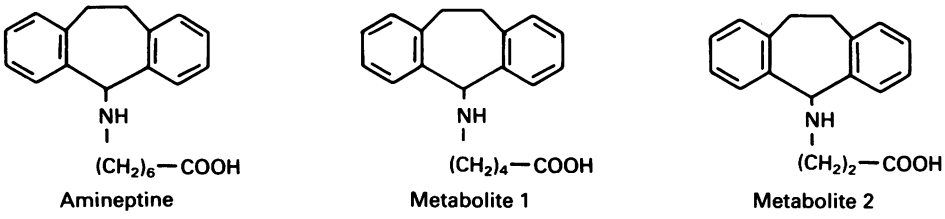

Figure 1 Chemical structure of amineptine and metabolites

Methods

Ex vivo *experiments*

Rats (Male Sprague Dawley CD COBS, Charles River, Italy, average weight 200 g) received i.p. amineptine (20 mg kg^{-1}) or saline twice daily for 15 days. Three days after the end of treatment animals were killed by decapitation, brain regions were quickly dissected and immediately used for synaptosomal experiments or stored at − 80°C until binding assays. For *in vitro* experiments either naive or saline-treated animals were used.

Binding assays

Crude membrane preparations used for all binding assays, were obtained as described by Bennett & Snyder (1976) and Nelson *et al.* (1978). The brain regions were homogenized in 50 vol of cold Tris-HCl buffer 0.05 M pH 7.4 using an ultra Turrax TP 18-10 (2 × 20 s) and centrifuged at 50,000 *g* for 10 min. The pellets were resuspended in cold Tris buffer, incubated at 37°C for 10 min and centrifuged twice more as above. For [^{3}H]-spiperone binding to dopamine$_2$-receptors (Creese *et al.*, 1978) incubation buffer (0.05 M Tris HCl, pH 7.1, 0.1% ascorbic acid, 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, final volume 0.5 ml) containing 2.5 mg of tissue (striatum) and [^{3}H]-spiperone (sp.act. 23 Ci $mmol^{-1}$, NEN) (0.010–1.4 nM) was incubated for 15 min at 37°C. Non-specific binding was determined in the presence of 1 μM (+)-butaclamol. For [^{3}H]-ketanserin binding to 5-hydroxytryptamine$_2$-(5-HT_2) receptors (Leysen *et al.*, 1982), incubation buffer (0.05 M Tris HCl, pH 7.7, final volume 1 ml) containing 5 mg of tissue (cortex) and [^{3}H]-ketanserin (sp.act. 77 Ci $mmol^{-1}$, NEN (0.3–5 nM) was incubated for 15 min at 37°C. Non-specific binding was determined in the presence of 1 μM methysergide. 5-HT_1 binding sites were measured by [^{3}H] 5-HT binding (Nelson *et al.*, 1978). Incubation buffer (0.05 M Tris HCl, pH 7.7, 0.1% ascorbic acid, 10 μM pargyline, 4 mM $CaCl_2$, final volume 1.0 ml) containing 10 mg of tissue (hippocampus) and [^{3}H]-5-HT (sp.act. 27 Ci $mmol^{-1}$, Radiochemical Centre) (0.10–15 nM) was incubated for 15 min at 37°C. Non-specific binding was determined in the presence of 10 μM unlabelled 5-HT. For [^{3}H]-dihyroalprenolol (DHA) binding to β-noradrenoceptors (Bylund & Snyder, 1976) incubation buffer (0.05 M Tris HCl, pH 7.7, 0.1% ascorbic acid, 10 μM pargyline, final volume 1 ml) containing 10 mg of tissue (cortex) and [^{3}H]-DHA (sp.act. 34 Ci $mmol^{-1}$, NEN) (0.9–7.5 nM) was incubated for 15 min at 25°C. Non-specific binding was determined in the presence of 1 μM (±)-propranolol. For [^{3}H]-prazosin (α_1-NA) (Greengrass & Bremner, 1979) binding incubation buffer (0.05 M Tris HCl, pH 7.7, 0.1% ascorbic acid, 10 μM pargyline, final volume 1 ml) containing 10 mg of tissue (cortex) and [^{3}H]-prazosin (sp.act. 18.8 Ci $mmol^{-1}$, NEN) (0.1–7 nM) was incubated for 30 min at 25°C. Non-specific binding was determined in the presence of 3 μM phentolamine. α_2-Noradrenoceptors were measured by [^{3}H]-clonidine binding (Greenberg *et al.*, 1976). Incubation buffer (0.05 M Tris HCl, pH 7.7, 0.1% ascorbic acid, 10 μM pargyline, final volume 1 ml) containing 10 mg of tissue (cortex) and [^{3}H]-clonidine (sp.act. 65.0 Ci $mmol^{-1}$, NEN) (1.0–15 nM) was incubated for 20 min at 25°C. Non-specific binding was determined in the presence of 100 μM noradrenaline (NA). *In vitro* affinities for dopamine agonist sites (Creese & Snyder, 1978) benzodiazepine- (Ehlert *et al.*, 1981), $GABA_A$-(Herschel & Baldessarini, 1979), histamine$_1$-(Tan Tran *et al.*, 1978) and antidepressant-(Raisman *et al.*, 1980; 1982) receptors were determined by using [^{3}H]-ADTN, [^{3}H]-flunitrazepam, [^{3}H]-GABA, [^{3}H-mepyramine], [^{3}H]-imipramine and [^{3}H]-desmethylimipramine, respectively. All ligands were obtained from NEN.

For all binding assays incubation was stopped by the addition of ice-cold Tris buffer followed by rapid vacuum filtration through Whatman GF/B glass fibre filters and three additional washes with Tris buffer. Filters were counted in 10 ml of Filter Count (Packard) scintillator in a Beckman liquid scintillation spectrometer L.S. 7500 with counting efficiency of

about 40%.

Binding parameters were calculated by non linear fitting of data from saturation experiments with 6–8 different concentrations of ^{3}H-ligands, or from inhibition experiments with 5 different inhibitor concentrations; using an HP 85 desk computer (Benfenati & Guardabasso, 1984).

Accumulation and release by brain synaptosomes

Purified synaptosomes were obtained from pooled cortex (5-HT and NA) or striata (dopamine) as described by Gray & Whittaker (1962) and diluted with Krebs-Henseleit buffer containing 0.25 mM pargyline to obtain a final protein concentration of 0.5–1 mg ml^{-1}. For accumulation studies, 0.6 ml samples were incubated at 30°C (or 4°C to assess passive diffusion) with 0.1 μM ^{3}H-monamines for 5 min. *In vitro* amineptine was added during a 5 min pre-incubation period. The reaction was stopped by cooling the tubes in ice and adding 0.5 ml of ice-chilled Krebs-Henseleit buffer. Samples were then filtered through cellulose nitrate filters (0.65 μM pore size; Società Italiana di Microfiltrazione), washed with Krebs-Henseleit buffer, dissolved in 5 ml of Atomlight (NEN) and counted for radioactivity in a Beckman LS 7500 liquid scintillator counter. Percentage accumulation inhibition was calculated on net values (30°C–4°C). The amineptine concentration giving 50% inhibition (IC_{50}) was determined by non-linear fitting of dose-response curves, with 5 drug concentrations.

For release experiments, synaptosome suspensions prelabelled by incubation with 0.1 μM ^{3}H-monamines for 15 min were centrifuged at 17,000 *g* for 10 min, and the pellets were gently resuspended in fresh Krebs-Henseleit buffer. Portions of 0.6 ml were preincubated 5 min at 37°C to allow equilibration. At the end of this period (0 time) drugs were added and the mixtures were then incubated for 20 min. Samples were filtered and counted as described for accumulation studies. % release was calculated as follows: (c.p.m. control 20 min − c.p.m. drug 20 min)/c.p.m. control 0 min × 100

For superfusion experiments, 0.8 ml portions of synaptosomes preloaded with 0.1 μM [^{3}H]-dopamine were filtered through cellulose nitrate filters. Filters were put at the bottom of superfusion chambers, and superfused with Krebs-Henseleit buffer at 0.5 ml min^{-1}. Effluent was collected in counting vials (every 5 min). At the end of superfusion (50 min) radioactivity remaining on the filters was also counted and used for calculation of total radioactivity present.

Data were calculated as % of radioactivity in each sample over total radioactivity present at the beginning of superfusion. More detailed information about the methods used in studies of monamine uptake and release is given elsewhere (Mennini *et al.*, 1978; 1981) [^{3}H]-NA was obtained from NEN, [^{3}H]-5-HT and [^{3}H]-dopamine from the Radiochemical Centre. Specific activities were as follows: [^{3}H]-NA 9.4 Ci mmol^{-1}; [^{3}H]-dopamine 9.0 Ci mmol^{-1}; [^{3}H]-5-HT 28.7 Ci mmol^{-1}.

Results

In vitro *experiments*

Amineptine and its two metabolites, up to a concentration of 10 μM, were unable to displace the following ligands from brain membrane preparations of naive rats: [^{3}H]-5-HT, [^{3}H]-spiperone (cortex and striatum), [^{3}H]-ATDN, [^{3}H]-prazosin, [^{3}H]-clonidine, [^{3}H]-DHA, [^{3}H]-GABA, [^{3}H]-mepyramine, [^{3}H]-flunitrazepam, [^{3}H]-imipramine and [^{3}H]-desmethylimipramine (data not shown).

In saline-treated animals, amineptine significantly inhibited [^{3}H]-dopamine accumulation (IC_{50} 1.4 μM) and at higher concentrations, [^{3}H]-NA accumulation (IC_{50} 10μM) (Table 1 top half). These effects were retained, with lower efficacy, by the two metabolites (data not shown). Amineptine 10 μM also released

Table 1 Effect of chronic amineptine on ^{3}H-monoamine accumulation (basal and amineptine-inhibited)

Treatment	*[^{3}H]-5-HT*		*[^{3}H]-NA*		*[^{3}H]-DA*	
	Control	*Amineptine (10 μM)*	*Control*	*Amineptine (10 μM)*	*Control*	*Amineptine (10 μM)*
Saline	1.77 ± 0.07	1.83 ± 0.07	0.367 ± 0.037	0.175 ± 0.01[a]	1.97 ± 0.03	0.33 ± 0.09[a]
Chronic	1.74 ± 0.06	1.72 ± 0.1	0.33 ± 0.02	0.153 ± 0.01[a]	0.68 ± 0.02[b]	0.557 ± 0.02[a]

Data (pmol min^{-1} mg^{-1} protein) are means ± s.d. of 4 replications.
Amineptine was given *in vivo* 20 mg kg^{-1} i.p., twice daily for 15 days; *in vitro* amineptine was tested at 10 μM final concentration.
[a]Different from respective control: [b]different from saline-treated; 2 ways ANOVA; $P < 0.05$ Tukey's test.
The final concentrations of ^{3}H-monoamines were: 5-HT and NA 0.1 μM; dopamine 0.05 μM.

Table 2 Effect of chronic amineptine on ^{3}H-monoamine release (basal and amineptine-stimulated)

	[^{3}H]-5-HT			*[^{3}H]-NA*			*[^{3}H]-DA*		
	Control 0 *min*	*Control* 20 *min*	*Amineptine* 10 μM 20 min	*Control* 0 min	*Control* 20 min	*Amineptine* 10 μM 20 min	*Control* 0 min	*Control* 20 min	*Amineptine* 10 μM 20 min
Saline	3.6 ± 0.5	2.9 ± 0.1	2.3 ± 0.1	1.41 ± 0.06	0.71 ± 0.05	0.80 ± 0.07	10.1 ± 0.3	6.7 ± 0.3	5.2 ± 0.4[b]
Chronic	3.1 ± 0.7	1.8 ± 0.7	2.0 ± 0.3	1.34 ± 0.09	0.69 ± 0.01	0.76 ± 0.02	7.4 ± 0.3[a]	4.9 ± 0.3[a]	3.5 ± 0.2[a,b]

Data (pmol mg^{-1} protein) are means ± s.d. of 4 replications.
Amineptine was given *in vivo* 20 mg kg^{-1} i.p., twice daily for 15 days.
In vitro amineptine was tested at 10 μM, final concentration.
[a]Different from saline-treated; [b]different from respective control 20 min; two ways ANOVA, $P < 0.01$, Tukey's test.

[^{3}H]-dopamine from preloaded synaptosomes but was inactive on [^{3}H]-NA release (Table 2 top half). At equimolar concentrations, metabolite 1 had no effect on ^{3}H-catecholamine release; metabolite 2 slightly (6.1 ± 0.2%) enhanced [^{3}H]-NA release and was only about half as active as the parent compound on [^{3}H]-dopamine release (7.4 ± 0.08%). Neither amineptine nor its metabolites influenced [^{3}H]-5-HT accumulation (Table 1 top half) or release (Table 2 top half) by brain synaptosomes of saline-treated rats. Table 3 shows that the releasing effect of amineptine on [^{3}H]-dopamine was potentiated by reserpine pretreatment, like that of (+)-amphetamine, reported for comparison. In fact, 1 μM amineptine did not increase [^{3}H]-dopamine release in normal synaptosomes, but it stimulated [^{3}H]-dopamine release in synaptosomes from reserpine-treated rats. The releasing effect of 10 μM amineptine was about half that of 1 μM amphetamine in normal and reserpinized synaptosomes.

Figure 2 shows that the effect of 10 μM amineptine on [^{3}H]-dopamine release seen in saline-treated rats is also evident in superfused synaptosomes, where interference due to reuptake inhibition is minimized.

Ex vivo *experiments*

Table 1 shows that chronic amineptine treatment did not affect basal [^{3}H]-5-HT accumulation, and confirms that, when added *in vitro* at 10 μM, it did not inhibit [^{3}H]-5-HT accumulation in rat cortical synaptosomes. Chronic amineptine treatment did not alter basal [^{3}H]-NA accumulation nor the sensitivity of the noradrenergic carrier to 10 μM amineptine added *in vitro* (about 50% inhibition), as shown in Table 1. Basal [^{3}H]-dopamine accumulation was significantly decreased in synaptosomes from rats chronically treated with amineptine (Table 1), as was the inhibiting activity of 10 μM amineptine added *in vitro*. While the IC_{50} of amineptine in saline-treated rats was 1.4 μM, in drug-treated animals only 30% inhibition of accumulation could be obtained with 30 μM amineptine (data not shown).

Figure 3 shows the kinetic characteristics of [^{3}H]-dopamine accumulation in striatal synaptosomes from saline or chronic amineptine-treated rats. The apparent affinities of the neuronal carrier for [^{3}H]-dopamine were similar in the two experimental groups (about 0.4 μM), but the maximum number of uptake sites was significantly lower in amineptine-treated animals (about 50% of control value).

Chronic amineptine treatment did not affect [^{3}H]-5-HT or [^{3}H]-NA release and did not seem to affect either basal or amineptine-stimulated [^{3}H]-dopamine release in incubated synaptosomes (Table 2). However, in superfusion experiments, a tendency to

Table 3 Effect of *in vitro* added amineptine on [^{3}H]-dopamine ([^{3}H]-DA) release from striatal synaptosomes

In vivo *treatment* *Drug*	*Concentration*	*% release induced during 20 min incubation* *Normal rats* A	*Reserpine-treated rats* B	B:A
(+)-Amphetamine	10^{-6} M	31 ± 3**	48 ± 2**	1.55
Amineptine	10^{-6} M	NS	13 ± 6**	—
Amineptine	10^{-5} M	15 ± 3**	23 ± 5**	1.53

[^{3}H]-dopamine release was studied in striatal synaptosome incubated *in vitro* as described in Methods. Reserpine-treated groups were injected with 10 mg kg^{-1} i.p. 18 h before the experiment. [^{3}H]-DA accumulation in synaptosomes from normal and reserpine-treated rats was 4,300 ± 8,500 and 30,000 ± 4,500 d.p.m. per sample, respectively ($P < 0.01$, Student's *t* test).
**$P < 0.01$ significantly different from controls (Duncan's test).

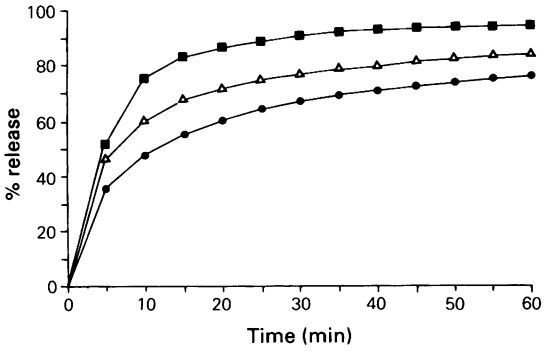

Figure 2 Effect of amineptine (10 μM) added *in vitro* or chronic amineptine treatment on [^{3}H]-dopamine release from superfused synaptosomes. Data were calculated as the cumulative percentage of total radioactivity (see Methods). x = time of superfusion (min); y = % of [^{3}H]-dopamine released. Each point is the mean of three replications, varying less than 10%. (●) = saline-treated rats; (■) saline + 10 μM amineptine *in vitro*; (△) = chronic amineptine-treated rats.

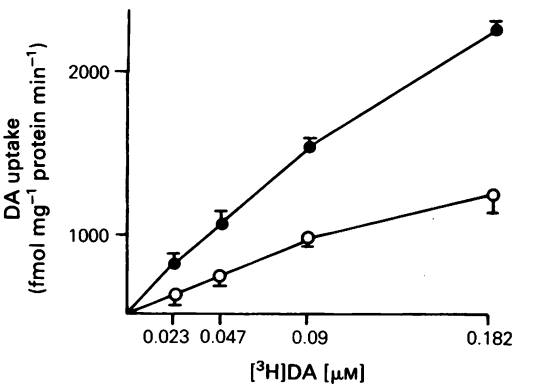

Figure 3 Effect of chronic amineptine treatment on [^{3}H]-dopamine accumulation. Each point is the mean ± s.d. of two replications: (●) = saline-treated rats; (○) = chronic amineptine-treated rats. The kinetic parameters, with the relative standard deviation, obtained by non-linear fitting of the data using a HP-85 computer were: Saline: V_{max} = 11939 ± 1136 fmol mg^{-1} protein min^{-1}; K_m = 0.43 ± 0.05 μM. Chronic amineptine: V_{max} = 5447 ± 2531 fmol mg^{-1} protein min^{-1}; $P < 0.05$ (Student's t test); K_m = 0.47 ± 0.28 μM.

higher spontaneous [^{3}H]-dopamine release was found in synaptosomes from chronically amineptine-treated animals (Figure 2).

Table 4 shows that chronic amineptine treatment significantly reduced the maximum number of binding sites for [^{3}H]-spiperone in striatum, as well as those for and α_2- and β-NA ligands in rat cortex. No significant effects were observed at 5-HT$_1$-, 5-HT$_2$- and α_1-NA binding sites.

Discussion

Amineptine and its two metabolites had no effect *in vitro* on a number of receptor sites. Of particular interest is the lack of effect on [^{3}H]-imipramine binding sites, in spite of the similarity in chemical structure. It is also important to consider that amineptine and its metabolites have no affinity for dopamine binding sites which means that direct agonism of the drug at dopamine receptors can be excluded. *In vitro* studies with rat brain synaptosomes have established that amineptine inhibits the accumulation of dopamine and, at ten times higher concentrations, that of NA, without affecting 5-HT accumulation. The inhibition exerted by amineptine was shared, but to a lesser extent by its two metabolites. Release of monoamines by amineptine was limited to dopamine, without any effect on NA and 5-HT. Again, the two metabolites were less effective than the parent drug.

These results point to amineptine as an indirect dopamine agonist. However, *in vitro* studies with synaptosomal preparations do not clarify whether a drug decreases amine accumulation inside the nerve terminal by reducing its uptake, increasing release, or both. Our results with superfused synaptosomes, where interference due to reuptake inhibition is minimized (Raiteri *et al.*, 1974), indicate that amineptine is a true dopamine releaser. Moreover, its effect of

Table 4 Effect of chronic amineptine on neurotransmitter receptor binding

Treatment		*5-HT$_1$*	*5-HT$_2$*	*DA$_2$*	*α_1-NA*	*α_2-NA*	β-NA
Saline	K_D	4.6 ± 0.9	0.8 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	2.4 ± 0.1	2.0 ± 0.0
	B_{max}	18.4 ± 3.3	15.2 ± 0.7	19.9 ± 0.9	6.7 ± 0.8	6.7 ± 0.1	12.4 ± 0.6
Chronic amineptine	K_D	4.1 ± 0.7	0.8 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	2.6 ± 0.1	2.2 ± 0.1
	B_{max}	18.9 ± 2.1	15.0 ± 0.3	15.3 ± 0.6*	6.3 ± 0.4	5.6 ± 0.2*	11.0 ± 0.9†

K_D (nM) and B_{max} (pmol g^{-1} tissue) are means ± s.d. of 4 animals and were calculated by non-linear fitting of binding data as described in methods.
*$P < 0.01$; †$P < 0.05$: Student's t test.

releasing dopamine is, like that of (+)-amphetamine, potentiated in synaptosomes from reserpine pretreated animals, suggesting that it acts on an extravescicular pool of dopamine. Whether it also acts by inhibiting the neuronal uptake carrier for dopamine is difficult to establish only on the basis of *in vitro* experiments; however, *in vivo* amineptine prevents the depletion of brain dopamine following 6-hydroxydopamine lesions, increases 3-methoxytyramine formation and reduces dopamine turnover (Garattini *et al.*, 1985), suggesting that inhibition of dopamine uptake had probably occurred (Ponzio *et al.*, unpublished).

Amineptine concentrations effective *in vitro* (1–10 μM) are in the range of those found in the brain of rats given pharmacologically active doses of the drug (20 mg kg^{-1}) (Sbarra *et al.*, 1979).

That amineptine acts as an (indirect) dopamine receptor agonist is also shown by the fact that repeated treatment induced down-regulation of dopamine receptor sites in striatum. The density of β-noradrenoceptors was also reduced in the rat cortex after repeated amineptine, a finding common to the majority of antidepressant agents (Charney *et al.*, 1981), as was the number of α_2-noradrenoceptors, a much more selective effect of antidepressants.

It is difficult to explain the effects of chronic amineptine on noradrenoceptors since, unlike amphetamine, *in vitro* it inhibits NA accumulation only at high concentrations and in *in vivo* it does not protect against 6-hydroxydopamine-induced NA depletion (Garattini *et al.*, 1985). Moreover, it apparently does not influence the noradrenergic at single doses (Ponzio, unpublished results). Whether the effect is related to stimulation of the dopaminergic system remains to be established.

An interesting observation arising from the present study is that the basal accumulation of dopamine in synaptosomes from rats chronically treated with amineptine was significantly decreased, as was the ability of *in vitro* added amineptine to inhibit dopamine accumulation. The reduced basal accumulation of dopamine could be due to enhanced release, as indicated by the fact that spontaneous release was increased in superfused synaptosomes from amineptine-treated animals. This result is at variance with those obtained by incubating synaptosomes, since spontaneous and amineptine-stimulated dopamine release were not modified by long-term treatment with the drug.

It might be argued that the enhanced release of [^{3}H]-dopamine in superfused synaptosomes is a consequence of the diminished [^{3}H]-dopamine accumulation after long-term amineptine treatment, which results in a considerably lower absolute amount of [^{3}H]-dopamine released. Further experiments are in progress to check this. It can be excluded that the decreased dopamine accumulation is caused by drug or metabolites present in the brain tissue, since treatment was suspended three days before the experiment in order to avoid interference due to residual drug concentrations.

It seems therefore that the presynaptic carrier for dopamine is modified by long-term amineptine treatment, resulting in an apparent reduction of the maximum number of uptake sites. While changes in sensitivity of pre- and postsynaptic receptors commonly occur after long-term drug treatment, (Creese & Sibley, 1981; Raiteri *et al.*, 1982) modifications of the neuronal uptake carrier have not been frequently reported. Chronic antidepressant treatment results in adaptive changes in the presynaptic monoaminergic system (Sugrue, 1981), principally in a compensatory variation in the turnover rate of these amines. It has been recently reported that dopamine reuptake is functionally coupled with tyrosine hydroxylase activity (Maura & Raiteri, 1982). Therefore the observed reduction in dopamine accumulation might reflect adaptive changes in presynaptic dopamine function following an amineptine-induced decrease in dopamine synthesis. It is of interest to note that *in vivo*, amineptine lost its effect on brain dopamine metabolism after three weeks of daily treatment, as indicated by the fact that a challenge with 40 mg kg^{-1} did not elicit the usual rise in striatal homovanillic acid (Garattini *et al.*, 1985).

In conclusion the results of this study are consistent with the hypothesis that amineptine increases dopaminergic transmission by presynaptic action, and indicate that long-term amineptine treatment induces adaptive modifications in pre- and postsynaptic mechanisms which may be responsible for its pharmacological effects.

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