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Effects of antidepressant drugs on dopamine uptake and metabolism

P. C. WALDMEIER, *Research Department, Pharmaceuticals Division, CIBA-GEIGY Ltd, Basle, Switzerland*

Since the introduction of the catecholamine hypothesis of depression, dopamine (DA) has not generally been considered to be of great importance in this group of illnesses, although some attempts have been made in this direction (Randrup et al 1975; Randrup & Braestrup 1977). In recent years, however, a number of antidepressants have been found to enhance dopaminergic function. Examples are nomifensine, an inhibitor of both noradrenaline (NA) and DA uptake (Hunt et al 1974; Schacht & Heptner 1974), bupropion (Cooper et al 1980) and amineptine (Offermeier et al 1977; Samanin et al 1977), which have been reported to inhibit DA uptake without affecting NA uptake. Amineptine also seems to induce DA release. On the other hand some drugs commonly used in the treatment of depression definitely possess antidopaminergic or neuroleptic-like effects, for example trazodone (Stefanini et al 1976) or trimipramine (Carlsson & Lindqvist 1978).

Moreover, recent data indicate an interaction of classical tricyclic antidepressant drugs with dopaminergic function after subacute or chronic treatment (Serra et al 1979; Chiodo & Antelman 1980; Naber et al 1980).

Therefore, a comparison of the effects of some classical antidepressants and more recently developed drugs on aspects of dopaminergic function under identical conditions seemed worthwhile. In this study, the acute effects on DA and NA uptake *in vivo* and on DA metabolism are reported.

Method

The test systems consisted of the reversal of the catecholamine depletion in the rat whole brain induced by α-methyl-*m*-tyrosine (AMMT) as a means of determining drug effects on catecholamine uptake (Fuller et al 1979), and the assessment of the effects on rat striatal homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), without and with pretreatment with spiperone. This latter test system, originally used by Shore (1976) who gave haloperidol rather than spiperone, modified by Fuller et al (1978), allows differentiation of amphetamine-like, methylphenidate-like and neuroleptic-like properties as well as DA agonistic effects. In general, the antidepressants were administered in a dose of 30 mg kg^{-1} i.p., except for nomifensine (10 and 30 mg kg^{-1} i.p.), bupropion (30–100 mg kg^{-1} i.p.) and oxaprotiline (30 and 60 mg kg^{-1} i.p.).

Apomorphine HBr and (+)-amphetamine-SO_4 were purchased from Siegfried AG (Zofingen, Switzerland). The following compounds were generously donated by the manufacturers: amineptine HCl (Lab. Servier, Orleans, France); butriptyline HCl (Ayerst Labs, New York, N.Y., USA); doxepine HCl (Chas. Pfizer & Co, Inc., New York, N.Y., USA); iprindol HCl (Wyeth Labs., Philadelphia, Penns., USA); mefexamide HCl (Lab. Anphar, Arceuil, France); nomifensine hydrogen maleinate (Farbwerke Hoechst, Frankfurt, FRG); spiperone (Janssens Pharmaceutica, Beerse, Belgium), trazodone HCl (Angelini, S.P.A., Rome, Italy). Amitriptyline HCl was synthesized in our Chemistry Department by Mr H. Blattner, befuraline HCl by Dr H. Allgeier, bupropion HCl by Dr R. Paioni, trimipramine maleate by Dr H. Schroeter, viloxazine oxalate by Dr R. Bernasconi, and zimelidine 2HCl by Prof. A. Marxer.

For the determination of the effects on DA metabolism, female Tif:RAIf (SPF) rats (Tierfarm Sisseln, Switzerland), 160–200 g, were treated with the drugs to be tested 1 h before decapitation. This was done in parallel with rats that had also received 0·5 mg kg^{-1} i.p. spiperone 1 h before the test drugs. Striatal HVA and DOPAC were isolated on Sephadex G 10 columns (Westerink & Korf 1976) and quantitated by h.p.l.c. with electrochemical detection (Waldmeier 1980).

For the determination of effects on DA and NA uptake, AMMT was administered as the methyl ester hydrochloride (40 mg kg^{-1} i.p.; Aldrich Co, Ltd, Milwaukee, Wis., USA) to female rats of the same strain and weights, which had been pretreated with the drugs to be tested for 15 min. The animals were killed 6 h later. NA and DA were isolated by butanol extraction essentially as described by Maickel et al (1968), and determined by automated fluorometry (Waldmeier et al 1974). Uptake inhibition in percent was calculated according to the formula (combination − AMMT)/(drug − AMMT) × 100.

Results and discussion

The effects of the drugs on HVA and DOPAC levels without and with pretreatment with spiperone are listed in Table 1. The results with apomorphine, (+)-amphetamine and methylphenidate are given for comparison. Significant increases in HVA were induced by amineptine, bupropion, clomipramine, mefexamide, nomifensine, opipramol, trazodone, trimipramine and zimelidine. With the exception of amineptine and mefexamide, these drugs concomitantly increased DOPAC levels.

When the animals were pretreated with spiperone only amineptine, bupropion, and nomifensine further enhanced the effect of the neuroleptic on DA metabolism. This indicates that their effect on striatal HVA and DOPAC levels are due to either inhibition of DA uptake or promotion of DA release. In contrast, the effects of those drugs which did not add to the effect of spiperone may be classified as neuroleptic-like.

A comparison of the effects of amineptine and amphet-

Table 1. Effects of antidepressant agents and reference compounds on rat striatal HVA and DOPAC levels without and with pretreatment with spiperone. Drugs were administered 1 h after 0·5 mg kg^{-1} i.p. spiperone, and the animals decapitated 1 h later. The means of the absolute control levels of HVA and DOPAC varied between 327–599 and 664–862 ng g^{-1}, respectively, and the corresponding values in the spiperone-treated rats were between 1539–2425 and 2482–3553 ng g^{-1}, respectively.

Drug	Dose mg kg^{-1} i.p.	(−)-Spiperone HVA % of control	(−)-Spiperone DOPAC % of control	(+)-Spiperone HVA % of control	(+)-Spiperone DOPAC % of control
Apomorphine	0·5[a]	43 ± 1** (5)	74 ± 3** (5)	82 ± 3 * (5)	83 ± 9 (5)
(+)-Amphetamine	10	132 ± 13 (5)	38 ± 3** (5)	123 ± 7 (5)	48 ± 4** (5)
Methylphenidate	10	161 ± 7** (5)	143 ± 2** (5)	179 ± 17** (4)	234 ± 24** (4)
Amineptine	30	171 ± 7** (5)	102 ± 9 (5)	154 ± 5** (5)	159 ± 7** (5)
Amitriptyline	30	122 ± 5 (5)	120 ± 4 * (4)	81 ± 4 * (5)	76 ± 4 * (4)
Befuraline	30	131 ± 8 (5)	84 ± 8 (5)	98 ± 4 (4)	89 ± 4 (4)
Bupropion	30	155 ± 9** (5)	145 ± 8** (4)	138 ± 8** (5)	139 ± 11 * (5)
Butriptyline	30	76 ± 11 (4)	88 ± 3 (5)	87 ± 15 (5)	80 ± 8 (5)
Clomipramine	30	138 ± 14** (5)	136 ± 7** (4)	83 ± 4** (4)	70 ± 8** (4)
Desipramine	30	109 ± 17 (5)	89 ± 5 (5)	89 ± 5 (5)	86 ± 5 (5)
Doxepine	30	118 ± 14 (5)	131 ± 15 (5)	78 ± 3** (5)	78 ± 2** (5)
Imipramine	30	101 ± 4 (5)	103 ± 7 (5)	71 ± 3** (5)	71 ± 8** (5)
Iprindol	30	99 ± 10 (4)	123 ± 10 (4)	74 ± 7 (5)	79 ± 5 (5)
Maprotiline	30	118 ± 12 (5)	118 ± 10 (5)	71 ± 18 (5)	65 ± 16 * (5)
Mefexamide	30	160 ± 14** (9)	116 ± 7 (10)	101 ± 8 (10)	89 ± 8 (10)
Nomifensine	10	157 ± 13** (5)	169 ± 14** (5)	207 ± 5** (5)	292 ± 22** (4)
Opipramol	30	201 ± 19** (5)	197 ± 16** (5)	87 ± 10 (5)	78 ± 9 (5)
(±)-Oxaprotiline	30	92 ± 7 (4)	98 ± 4 (5)	92 ± 8 (5)	84 ± 6 (5)
	60	91 ± 18 (5)	100 ± 6 (5)	87 ± 15 (5)	76 ± 3** (5)
(+)-Oxaprotiline	30	86 ± 4 (3)	99 ± 2 (4)	94 ± 1 (4)	86 ± 4 (5)
(−)-Oxaprotiline	30	86 ± 4 (4)	98 ± 1 (5)	95 ± 5 (5)	100 ± 5 (5)
Trazodone	30	200 ± 16** (5)	176 ± 13** (5)	80 ± 5 (5)	74 ± 1 (5)
Trimipramine	30	244 ± 42** (4)	218 ± 26** (4)	76 ± 9 (5)	76 ± 11 (5)
Viloxazine	30	103 ± 5 (5)	87 ± 10 (5)	94 ± 4 (5)	80 ± 2 (5)
Zimelidine	30	158 ± 14 * (5)	149 ± 9 * (5)	87 ± 8 (4)	82 ± 4 (5)

[a] Apomorphine was given twice, 1 h and 30 min before death.
* $P < 0·05$, ** $P < 0·01$ vs appropriate controls (Dunnett's test).

amine suggests some similarities: amphetamine increased HVA and lowered DOPAC, and amineptine produced a large increase in HVA without altering DOPAC. This could be merely a quantitative difference. However, whereas the pattern with amphetamine remained the same in the spiperone-pretreated rats, amineptine enhanced the effect of the neuroleptic on both metabolites, resembling methylphenidate or nomifensine in this respect. Such an intermediate pattern with amineptine has also been reported by Samanin et al (1977) on the basis of pharmacological investigations and effects on acetylcholine levels.

Many of the drugs slightly attenuated the effect of spiperone on HVA and DOPAC, in some cases significantly. Such an effect was not only shown by drugs which, when given alone, did not alter DA metabolism (butriptyline, imipramine, iprindol, maprotiline, oxaprotiline, viloxazine) but also by some of those which exhibited slight, just significant or non-significant increases (amitriptyline, clomipramine, doxepine, zimelidine) as well as by those with overt neuroleptic-like effects (opipramol, trazodone, trimipramine). Since 0·5 mg kg^{-1} i.p. spiperone produces a maximal effect on DA metabolism, an interference with spiperone bioavailability is not a likely explanation of this phenomenon. A plausible explanation cannot be given without further investigation.

In the second series of experiments, the effects of classical tricyclic drugs and also of those compounds which had increased DA metabolism, on the AMMT-induced depletion of brain NA and DA were investigated. From the results (Table 2) it is clearly evident that the only drug in the whole series that was able to significantly attenuate the effect of AMMT on brain DA concentrations was nomifensine. In contrast to the reports by Offermeier et al (1977) and Samanin et al (1977) on amineptine, and by Cooper et al (1980) on bupropion, no DA uptake inhibitory effects of these drugs could be observed in this test system. Bupropion, in particular, was ineffective even at the high dose of 100 mg kg^{-1} i.p.

This discrepancy might be explained by the use of different test systems: Samanin et al (1977) and Cooper et al (1980) used 6-hydroxydopamine to deplete brain DA and observed significant antagonism with amphetamine, amineptine and bupropion. A similar effect of nomifensine had been reported before (Samanin et al 1975). For the interpretation of the results with this test system, the possibility should be considered that DA-releasing agents might also release freshly captured 6-hydroxydopamine before it can damage the DA neuron; this would also lead to a reduced DA depletion and thus simulate DA uptake inhibition.

Table 2. Effects of antidepressants on NA and DA uptake in rat brain in vivo. Drugs were administered 15 min before α-methyl-*m*-tyrosine methyl-ester hydrochloride (AMMT; 40 mg kg^{-1} i.p.) and the animals (n = 5) decapitated 6 h thereafter. NA and DA were determined in rat whole brain.

	Dose	−AMMT		+AMMT		% Upt. inhib.	
	mg kg^{-1} i.p.	NA ng g^{-1}	DA ng g^{-1}	NA ng g^{-1}	DA ng g^{-1}	NA	DA
Controls		413 ± 19	617 ± 7	136 ± 7	372 ± 27		
Methylphenidate	10	381 ± 13	571 ± 9*	155 ± 5	313 ± 4	8	−30
Controls		422 ± 8	801 ± 12	127 ± 5	441 ± 25		
Nomifensine	10	400 ± 8	763 ± 8	352 ± 4**	497 ± 9	82	17
Controls		368 ± 6	768 ± 13	103 ± 4	451 ± 21		
Nomifensine	30	326 ± 6*	731 ± 16	343 ± 18**	605 ± 17**	107	59
Controls		290 ± 17	555 ± 46	83 ± 3	278 ± 12		
Clomipramine	30	273 ± 10	482 ± 18	159 ± 5**	184 ± 18**	40	−46
Mefexamide	30	272 ± 8	483 ± 12	62 ± 2**	226 ± 8**	−11	−25
Trimipramine	30	276 ± 11	533 ± 26	60 ± 2**	203 ± 7**	−12	−29
Controls		362 ± 8	598 ± 20	99 ± 4	327 ± 10		
Amineptine	30	357 ± 12	624 ± 11	110 ± 4	340 ± 12	4	4
Amitriptyline	30	366 ± 3	628 ± 8	215 ± 12**	354 ± 35	43	9
Befuraline	30	373 ± 12	607 ± 18	160 ± 3**	301 ± 8	22	−9
Imipramine	30	392 ± 7	644 ± 15	293 ± 12**	306 ± 30	66	−6
Opipramol	30	368 ± 8	642 ± 7	103 ± 6	324 ± 15	1	−1
Controls		261 ± 6	568 ± 11	73 ± 4	313 ± 9		
Bupropion	50	247 ± 6	551 ± 13	92 ± 11	295 ± 33	11	−8
Desipramine	30	256 ± 3	517 ± 9 *	215 ± 4**	208 ± 7**	76	−51
Doxepine	30	246 ± 8	494 ± 9**	147 ± 8**	331 ± 33	43	10
Trazodone	30	264 ± 13	544 ± 7	66 ± 3	241 ± 3	−4	−31
Zimelidine	30	259 ± 8	513 ± 14**	89 ± 6	229 ± 17**	9	−42
Controls		301 ± 10	527 ± 17	89 ± 3	291 ± 11		
Bupropion	100	261 ± 10	487 ± 9	105 ± 7	324 ± 14	9	17

* $P < 0.05$, ** $P < 0.01$ vs appropriate controls (Dunnett's test).

Cooper et al (1980) also used AMMT, but they administered bupropion 5 h after the depleting agent. This schedule may not be adequate for the assessment of uptake inhibitory properties. The disappearance of α-methyl-*m*-tyramine from the brain after AMMT administration reflects DA turnover (Dorris & Shore 1974; Fuller & Perry 1978). The change in its levels is reciprocal to that of DA (Dorris & Shore 1971). Thus, if DA turnover or utilization is increased, α-methyl-*m*-tyramine levels decrease and DA levels recover faster. This seems to be a more likely explanation of the effect of bupropion observed by Cooper et al (1980) than uptake inhibition, since it is difficult to understand how the latter could be effective if the drug is given 5 h after the depletor.

The test system used in the present study discriminated between nomifensine on the one hand and amineptine and bupropion on the other. It is at present not clear whether it is less sensitive or more specific than the 6-hydroxydopamine model.

Many of the compounds that increased DA metabolism (Table 1) enhanced the DA depletion by AMMT (Table 2). Exceptions are nomifensine, amineptine and bupropion. On the other hand, desipramine, which did not increase DA metabolism as measured by HVA/DOPAC concentrations, also enhanced DA depletion by AMMT.

If given long after AMMT, DA antagonists and releasers increase α-methyl-*m*-tyramine disappearance (Dorris & Shore 1974) and would correspondingly be expected to enhance DA recovery (see above). However, if treatment with drugs enhancing DA utilization precedes administration of AMMT, increased DA demand and depletion by α-methyl-*m*-tyramine might occur, resulting in an enhanced depletion of the endogenous amine. Therefore, such an effect of some of the antidepressants might reflect an increase in DA utilization. Its absence in the cases of amineptine and bupropion might indicate that those drugs possess both uptake-inhibiting and -releasing properties, which in this test system seem to counteract each other.

In conclusion, the results of this comparative investigation of antidepressant drugs with respect to their acute effects on DA uptake and metabolism suggest that there are four types of compounds in this series:

(1) those which do not possess a measurable effect on the parameters (befuraline, butriptyline, desipramine, doxepine, imipramine, iprindol, maprotiline, oxaprotiline, and viloxazine); (2) those which possess neuroleptic-like properties to a slight or moderate degree (clomipramine, mefexamide, opipramol, trazodone, trimipramine, zimelidine); (3) those which possess DA releasing and perhaps also uptake inhibiting properties (amineptine, bupropion); (4) one drug which only affects DA uptake (nomifensine).

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Does ethyl-β-carboline-3-carboxylate interact with mouse brain benzodiazepine receptors in vivo?

T. MENNINI*, S. COTECCHIA, S. CACCIA, S. GARATTINI, *Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 Milan, Italy*

β-Carboline-3-carboxylic acid ethyl ester (β-CEE) has been recently isolated from human urine and brain extracts from different animal species (Braestrup et al 1980). On account of its selective affinity for benzodiazepine (BDZ) binding sites, in the nanomolar range, β-CEE has been related to an endogenous ligand for BDZ receptors in the brain (Braestrup et al 1980).

Experimental data in animals indicate that β-CEE has pharmacological activity opposite to that of diazepam (Jones & Oakley 1981), it produces a dose-dependent increase of the 50% protective dose (PD50) of diazepam against leptazol (pentetrazol)-induced convulsions and it lowers the seizure threshold in mice (Tenen & Hirsch 1980). Recently, Hirsch & Lydigsen (1981) reported that β-CEE displaces [^{3}H]flunitrazepam from mouse brain benzodiazepine receptors in vivo. In the present study we investigated whether β-CEE displaces [^{3}H]diazepam bound to mouse brain, when given at doses required to antagonize the antileptazol effect of diazepam.

Treatment schedules were as reported by Tenen & Hirsch (1980) and the presence of convulsions was evaluated in a separate group of mice. Female CD mice (Charles River, Italy), 25–30 g, were pre-treated with either 0·9% NaCl (saline) or β-CEE, 10 mg kg^{-1} intravenously, and 5 min later intraperitoneally with vehicle or diazepam at two different doses. Twenty minutes later, the animals were injected intravenously with 35 mg kg^{-1} of leptazol or 25 μCi [^{3}H]diazepam (S.A. 87.6 Ci mmol^{-1}, New England Nuclear). In vivo [^{3}H]diazepam binding was assayed according to Williamson et al (1978). Mice were injected in the lateral tail vein with 25 μCi of [^{3}H]diazepam in 0·2 ml of saline, and decapitated 1 min after injection. Brain and cerebellum were immediately removed, hemisected and homogenized in 50 volumes of ice-cold Tris HCl buffer (50 nM, pH 7·4) using an Ultra-Turrax TP18-10 (20 s, full speed). One half of the tissue was homogenized in Tris-HCl buffer containing 3 μM diazepam, and incubated at 0 °C for at least 30 min, to determine non-specific binding. 0·5 ml aliquots of the tissue homogenate were filtered through Whatman GF/B filters, washed twice on the filters with 5 ml of ice-cold Tris-HCl buffer and counted in 10 ml Dioxane scintillator (Supelchem).

Percentage of specific binding is defined as the amount of radioactivity specifically retained on the filter divided by the total amount of radioactivity present in the tissue aliquot (homogenate) × 100. In these conditions, β-CEE did not significantly affect brain diazepam levels (Table 1), indicating that its antagonism of diazepam's effect cannot be explained on pharmacokinetic grounds.

As shown in Table 2, diazepam produced dose-dependent occupancy of BDZ binding sites in mouse brain.

* Correspondence.