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INHIBITION OF [^{3}H]DOPAMINE ACCUMULATION IN RAT STRIATAL SYNAPTOSOMES BY PSYCHOTROPIC DRUGS

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Abstract—The accumulation of ^{3}H-labelled dopamine ([^{3}H]DA) in crude synaptosomal fractions from rat corpus striatum was found to be time and temperature dependent. The accumulation was saturable with a K_m value of 105 nM and was inhibited noncompetitively by benztropine. Series of thymoleptics, neuroleptics, stimulants, and miscellaneous compounds were tested for inhibiting properties in the system. The concentration-response curves were parallel to that of benztropine. The thymoleptics were rather weak inhibitors with potencies not comparable to their inhibition of either noradrenaline or serotonin uptake. Only a few neuroleptics (diphenylbutylpiperidines) showed potencies comparable to benztropine, whereas most of the neuroleptics were rather weak. The isomers of the thioxanthenes were almost equipotent, and the potencies of the neuroleptics claimed to induce few extrapyramidal side effects varied considerably but covered the same concentrations as the neuroleptics normally inducing these side effects. The stimulants were all rather potent, whereas anti-cholinergics were very weak and GABA uptake inhibitors were without effect. It is concluded, that the accumulation of [^{3}H]DA clearly deviates in steric requirements from the uptake of noradrenaline, serotonin and γ-aminobutyric acid, and probably represents the difference between DA uptake and release. Furthermore, the test does not seem to be predictive for clinical effects of antidepressants and neuroleptics.

A common feature of the tricyclic antidepressants is their ability to inhibit the reuptake mechanism for either noradrenaline (NA) or 5-hydroxytryptamine (serotonin, 5HT), and the hypothesis was advocated that facilitation of NA and 5HT transmission was related to increase in drive and to mood elevation, respectively [1–6]. On the other hand, the effect of neuroleptics in schizophrenia is normally associated with their antagonism of dopaminergic (DA) systems in the brain [7–12]. However, DA could play some role in depression but the effect of thymoleptics on DA uptake has only received little attention compared with the experiments involving NA and 5HT [13–16]. Furthermore, the contribution of the DA uptake inhibiting or releasing properties of neuroleptics to their therapeutic mode of action has not been so intensively investigated [13, 14, 17–19]. The present investigation deals with the effect of a series of thymoleptics, a series of neuroleptics and some compounds with miscellaneous effect on the accumulation of ^{3}H-labelled DA in rat striatal synaptosomes. It is meant as a contribution to the discussion of the possible involvement of DA uptake inhibition in the action of thymoleptics and neuroleptics in affective disorders.

MATERIALS AND METHODS

Male rats (Wistar Af/Han/Mol (Han 67), SPF, 200–250 g) were killed by a blow to their heads, exsanguinated and their brains removed. The brain was placed on a precooled glassplate, and the two corpora striata were dissected out and gently homogenized in 40 vol. of ice-cold 0.32 M of sucrose containing 1 mM of nialamide using a hand homogenizer with Teflon pestle (Potter–Elvehjem type, Thomas Tissue Grinder, clearance 0.004–0.006 inch, ten strokes up and down). [20]. The P_2 fraction (crude synaptosomal fraction) was obtained by centrifugation (600 *g*, 10 min 25000 *g*, 55 min) and suspended in 40 vol. of a modified Krebs–Ringer–phosphate buffer, pH 7.4 (122 mM NaCl, 4.8 mM KCl, 972 μM $CaCl_2$, 1.2 mM $MgSO_4$, 12.7 mM Na_2HPO_4, 3.0 mM NaH_2PO_4, 162 μM EDTA-Na_2, 1.14 mM ascorbic acid and 10.1 mM glucose, oxygenated with pure oxygen for 10 min before use). To 200 μl of the crude synaptosomal fraction on ice were added 3700 μl modified Krebs–Ringer–phosphate buffer—containing test compounds. After a preincubation at 37° for 5 min 100 μl of [^{3}H]DA (3,4-dihydroxyphenylethylamine (ethyl-1-[^{3}H](N)), spc. act. 8.95 Ci/m-mole-NEN, final conc. 12.5 nM) were added and the samples were incubated for 5 min at 37°. The incubation was terminated by filtering the samples under vacuum through Millipore filters (HAWP 02500, 0.45 μ, composed of a mixture of cellulose acetate and cellulose nitrate) with a wash of 5 ml buffer containing 10 μM unlabelled DA. After solubilizing the filters in 1 ml of cellosolve the radioactivity was determined by liquid scintillation counting after the addition of 10 ml of Instagel® (Packard). The unspecific binding of [^{3}H]DA was determined by incubating control samples on ice instead of at 37°. IC_{50} values were derived from concentration–response curves as the concentrations causing 50 per cent inhibition of the active [^{3}H]DA uptake (uptake at 37°-uptake at 0° = 100 per cent uptake). Normally four to five concentrations each in triplicate were used for constructing the concentration–response curves,

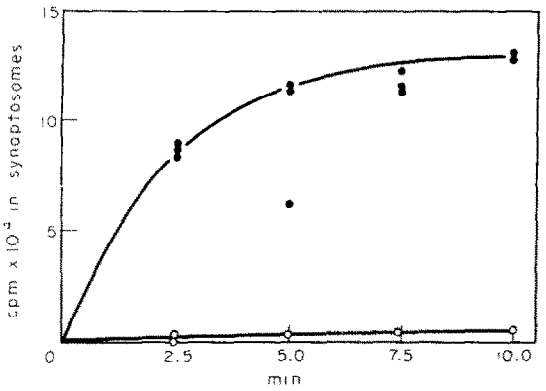

Fig. 1. Time course of the accumulation of [^{3}H]DA into rat striatal synaptosomes. Synaptosomes were incubated in 12.5 nM of [^{3}H]DA at 37° (●) or 0° (○) for 2.5, 5, 7.5, and 10 min. Accumulation is expressed in cpm, and each point represents a single determination.

and the IC_{50} values appearing in the tables are the means from at least two curves.

In order to characterize the system different concentrations of [^{3}H]DA and different incubation times were used. In the Lineweaver and Burk plot the lines were calculated according to the method of least squares.

RESULTS

Tritium labelled DA (12.5 nM) accumulated time and temperature dependently in striatal synaptosomes, but the rate of accumulation decreased with time (Fig. 1). The Lineweaver and Burk plot (Fig. 2) shows that the accumulation was saturable with a K_m value of 105 nM. Benztropine inhibited the accumulation of [^{3}H]DA non-competitively (Fig. 2) with a K_i value of 1.5×10^{-7} M. The accumulation of [^{3}H]DA was inhibited concentration dependently by all the drugs tested, except those with IC_{50} values $> 100\ \mu$M (Table 1). In all cases a significant concentration–effect relationship was obtained, and most likely the inhibition was non-competitive since all the concentration–effect curves were parallel to that of benztropine (Fig. 3).

The thymoleptics (Table 1) were all rather weak inhibitors of DA accumulation being 20 to > 900 times weaker than benztropine. In the tricyclic series there were no difference between the potencies of the monomethylamino and the corresponding dimethylamino derivatives. The specific 5HT uptake inhibitor, citalopram (Lu 10-171) and its desmethyl- and didesmethyl metabolites (Lu 11-109 and Lu 11-161) were all weak inhibitors of DA accumulation, whereas the *N*-oxide metabolite (Lu 11-305) was without effect [21, 22].

The potent NA-uptake inhibiting compounds, talopram (Lu 3-010) and talsupram (Lu 5-003) were also weak inhibitors of DA-accumulation [23–25].

Generally, the neuroleptics (Table 1) showed weak inhibition of DA-accumulation. However, the diphenylbutylpiperidines were equipotent with benztropine, whereas sulpiride and metoclopramide were without effect. In the thioxanthene series no difference between the *cis* and *trans* isomers of the thioxanthenes were observed.

The stimulants, *d*-amphetamine, methylphenidate and cocaine, were all very active, approximately half as active as benztropine, whereas nomifensine was found twice as active as benztropine. The DA receptor agonist, apomorphine, was a rather weak inhibitor of DA accumulation. The anticholinergic compounds, atropine and scopolamine were both extremely weak, and the GABA (γ-

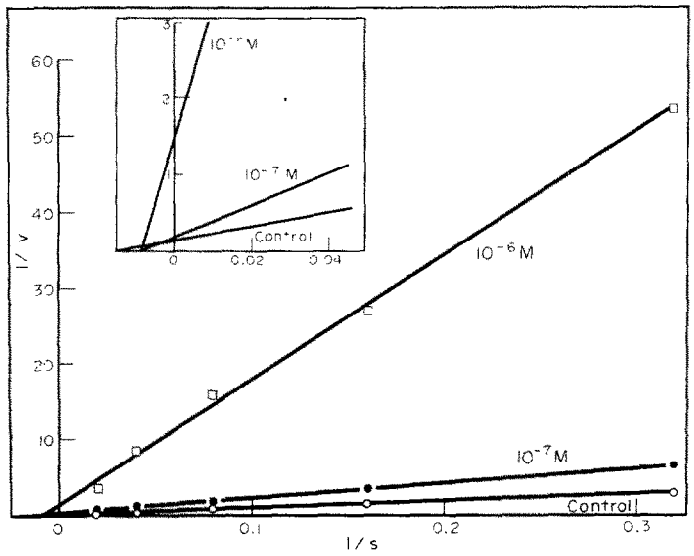

Fig. 2. Double reciprocal plots (Lineweaver and Burk) of the accumulation rates (v, [^{3}H]DA 10^{-5} cpm/5 min ordinate) versus extrasynaptosomal [^{3}H]DA concentration (s, nM) in a medium without (○) or with benztropine present in concentrations of 10^{-6} (□) and 10^{-7} M (●). Synaptosomes were incubated for 5 min at 37°. Each point represent the mean ± S.D. (vertical bars) of triple determinations. Where no bars are present S.D. were less than the symbols used for showing the mean. From each intersect on the abcissa (1/Km) K_m values were calculated and the mean ± S.D. determined giving $K_m = 105 \pm 24$ nM. Insert illustrates the lowest parts of the lines in greater magnification.

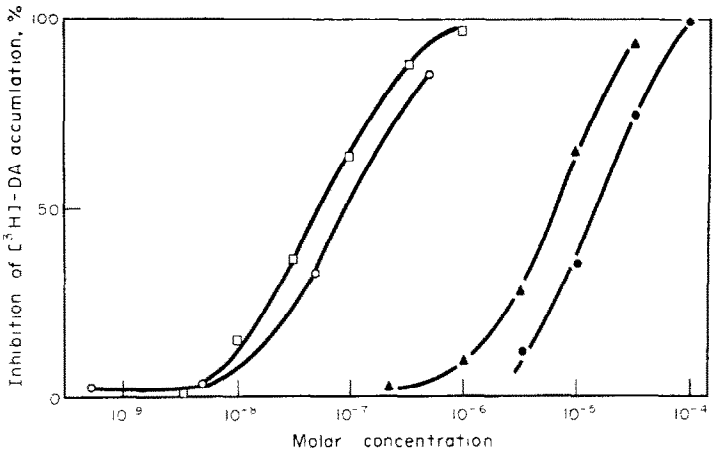

Fig. 3. Typical concentration–response curves for drug induced inhibition of [^{3}H]DA accumulation in rat striatal synaptosomes. Each point is the mean of three determinations. □ nomifensine, ○ benztropine, ▲ *cis* (z)-chlorprothixene, ● imipramine.

aminobutyric acid) uptake inhibitors, nipecotic acid and guvacine, and GABA itself had no effect on DA accumulation.

DISCUSSION

The accumulation of [^{3}H]DA in rat striatal synaptosomes was time and temperature dependent and obeyed saturation kinetics with a K_m value of 105 nM in agreement with values found by Hunt *et al.* [26], Halaris *et al.* [15], Snyder and Coyle [27], and Tuomistro *et al.* [28]. The non-competitive nature of the inhibition by benztropine was also found by Horn *et al.* [13], who showed this also to be true for tricyclic thymoleptics and neuroleptics. The inhibition of [^{3}H]DA accumulation by thymoleptics and neuroleptics in the present study was most likely also non-competitive since the concentration–effect curves for these compounds were parallel to that of benztropine.

The present results do not distinguish between uptake inhibition and release, but merely express the sum of these two effects, which is designated inhibition of accumulation. However, the effects *in vivo* of release and uptake inhibition are quite different, e.g. the uptake inhibitor benztropine by itself does not induce stereotyped behaviour whereas *d*-amphetamine and methylphenidate, which are regarded as releasing compounds (see below), induce a profound stereotyped behaviour via activation of DA receptors [30, 31]. Neuroleptics have been shown to increase stimulation induced overflow of [^{3}H]DA from rat brain slices, and the spontaneous release of preloaded [^{3}H]DA from synaptosomes in much lower concentrations than those inhibiting DA uptake [19, 17]. The latter observation was found to be true for a great number of drugs, including thymoleptics and neuroleptics, and it was concluded that before a drug can be designated as an uptake inhibitor, the concentration–effect curve for inhibition of accumulation should be distinctly to the left of the concentration–effect curve for release [16, 29]. Therefore further biochemical or pharmacological studies are needed to clarify whether release or uptake inhibition is most important for a given compound showing DA accumulation inhibiting properties in the present study.

The thymoleptics were all weak inhibitors of DA-accumulation as found earlier for tricyclic thymoleptics by Horn *et al.* [13]. In the tricyclic series there was no difference between the potencies of the monomethylamino and the corresponding dimethylamino derivatives in agreement with results obtained by Halaris *et al.* [14, 15], but in contrast to those of Horn *et al.* [13], who found amitriptyline and imipramine to be more potent than nortriptyline and desipramine, respectively.

In the tricyclic series the monomethylamino derivatives are normally more potent inhibitors of NA uptake than their corresponding dimethylamino derivatives, whereas the reverse is true when inhibition of 5HT uptake is considered [1, 2, 21]. In the bicyclic series compounds which exclusively inhibit 5HT uptake (Lu 10-171, Lu 11-109, Lu 11-161, and Lu 11-305) [21] or NA uptake (Lu 3-010 and Lu 5-003) are found [23–25]. However, these structure-activity relations are not found concerning DA accumulation, which shows that the steric requirements for NA, 5HT and DA accumulation are quite different.

Generally, the neuroleptics were weak inhibitors of DA-accumulation. However, the diphenylbutylpiperidines were equipotent with benztropine. The high potency of pimozide has earlier been described by Halaris and Freedman [14]. The figures for inhibition of DA uptake by chlorpromazine, haloperidol and pimozide obtained by Seeman and Lee [17] are rather high compared to the present results. The higher concentration of [^{3}H]DA used by Seeman and Lee (2×10^{-6} M) probably does not explain the differences since the inhibition by the neuroleptics is supposed to be non-competitive and therefore K_i values equal IC_{50} values [32]. However, Seeman and Lee used 20 min of incubation, stopped the reaction by centrifugation and washed the pellet in DA free medium. These differences could eventually contribute to the differences in potencies.

Promethazine, aceperone and metoclopramide, which are without antipsychotic effect are among the weakest neuroleptics tested. However, also neuroleptics with antipsychotic effect, clozapine

Table 1. Inhibition of [^{3}H]DA accumulation in rat striatal synaptosomes. The IC_{50} values are means of at least two experiments, not deviating more than 30 per cent. For each concentration–effect curve 4–5 concentration (each in triplicate) covering approximately two decades of concentration were used. In all instances a significant concentration–effect relationship was obtained

		Compound	IC_{50} μM
		Benztropine	0.11
Thymoleptics	Tricyclics	imipramine	18
		desipramine	9.1
		amitriptyline	5.4
		nortriptyline	3.6
		N-methylprotriptyline	3.9
		protriptyline	3.3
		chlorimipramine	4.3
		chlordesipramine	2.2
		melitracen	8.2
		litracen	8.0
		iprindol	14
		opipramol	3.0
		doxepin	13
		trimipramine	6.6
		butriptyline	5.5
		dibenzepine	> 100
		danitracene	65
	Tetracyclic	maprothilene	10
	Bicyclics	lu 10-171 (citalopram)	41
		lu 11-109	26
		lu 11-161	12
		lu 11-305	> 100
		lu 3-010 (talopram)	44
		lu 5-003 (talsupram)	9.3
		viloxazine	56
Neuroleptics	Thioxanthenes	cis (Z)-chlorprothixene	6.3
		trans (E)-chlorprothixene	2.8
		cis (Z)-flupenthixol	1.4
		trans (E)-flupenthixol	2.1
		cis (Z)-clopenthixol	0.39
		trans (E)-clopenthixol	0.83
		teflutixol	5.0
		piflutixol	1.6
	Phenothiazines	chlorpromazine	5.6
		promethazine	15
		trifluoperazine	3.5
		perphenazine	1.5
		fluphenazine	4.6
		thioridazine	2.6
	Butyrophenones	haloperidol	4.0
		aceperon	39
		spiroperidol	5.5
	Diphenylbutyl-piperidines	pimozide	0.07
		fluspirilene	0.26
		penfluridol	0.17
		clozapine	24
		sulpiride	> 100
		(±)-butaclamol	67
		metoclopramide	> 100
Stimulants		amphetamine	0.24
		methylphenidate	0.20
		cocaine	0.31
		nomifensine	0.05
		apomorphine	7.6
Anticholinergics		atropine	65
		scopolamine	270
GABA-ergics		nipecotic acid	> 100
		guvacine	> 100
		GABA	> 100

and butaclamol, are found much weaker than rest of the neuroleptics.

Among the thioxanthenes clopenthixol was most active. However, no difference between the *cis* and *trans* isomers of the thioxanthenes were observed, in contrast to their DA receptor blocking effect [9–12]. The four neuroleptics normally claimed not to induce extrapyramidal side effects in man, thioridazine, chlorprothixene, clozapine, and sulpiride were 24, 57, 218 and > 900 times weaker than benztropine, respectively [33–36]. From this it follows, that the occurence of extrapyramidal side effects can be ascribed neither to the presence nor to the lack of DA accumulation inhibiting properties of neuroleptics. The present test does not seem to be predictive for clinical effect, since both thymoleptics and neuroleptics are active in almost the same concentration range. Furthermore, in the neuroleptic series the antipsychotic and the DA receptor blocking effects do not correspond to the DA accumulation inhibiting properties, which further strengthens this conclusion. The stimulants, *d*-amphetamine, methylphenidate and cocaine, were all very active inhibitors of DA-accumulation, approximately half as active as benztropine. *d*-Amphetamine and methylphenidate have been classified as DA-releasing compounds influencing the newly synthesized pool and the storage pool, respectively [37]. In this type of experiments cocaine corresponds to the methylphenidate group [38]. However, in uptake–release studies cocaine was the only compound tested which did not induce DA-release, and cocaine was therefore claimed to be a pure DA-uptake inhibitor [16]. Biochemically *d*-amphetamine has been shown strongly to affect both [^{3}H]DA uptake and release in synaptosomes [39]. Nomifensine was found twice as active as benztropine in agreement with results obtained by Hunt *et al.* [26]. Nomifensine has been classified as an antidepressant but newer results seem to indicate that nomifensine is a releasing compound of the methylphenidate type [40, 41].

The above discussion shows that the stimulants excert their action through either DA release or DA uptake, or probably both. Whatever the mechanism is, the net result in the present test is a rather potent inhibition of the accumulation of DA by all four drugs. In contrast to the four stimulants just discussed, the DA receptor agonist, apomorphine, is a rather weak inhibitor of DA accumulation [42]. The lack of effect on DA accumulation by GABA and GABA uptake inhibitors indicate that accumulation of DA and GABA are clearly different in steric requirements.

In conclusion, the accumulation of [^{3}H]DA in synaptosomes clearly deviates in steric requirements from the uptake of NA, 5HT, and GABA. The results with the stimulants seem to indicate that compounds which either release DA or inhibit its reuptake will be potent in the present model. Furthermore, the test does not seem to be predictive for antidepressant effect of thymoleptics or for antipsychotic effect or extrapyramidal side effects of neuroleptics.

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