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ARTEFACTUAL INHIBITION OF DOPAMINE UPTAKE BY PSYCHOTROPIC DRUGS ON STRIATAL SYNAPTOSOMAL PREPARATION

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Abstract—On striatal synaptosomal preparations, using a double labelling test, numerous antidepressant drugs demonstrated an inhibitory effect on [^{3}H]DA uptake at the same high concentrations producing a [^{14}C]DA release. This releasing effect was also shared by non-antidepressant agents and was observed on synaptosomes preloaded with [^{3}H]5HT. The imipramine-induced release of DA was not modified by increasing concentration of K^+, by decreasing concentration of Na^+, by deleting Ca^{2+} from the incubation medium, or by blocking the catecholamine uptake systems with nomifensine or cocaine. The imipramine effect was reversible and was possibly initiated by a transient physico-chemical modification of the synaptosomal membrane. It was concluded that the DA uptake carrier is probably not involved in the effect of these drugs.

Various antidepressants have been claimed to inhibit the dopamine (DA) uptake by dopaminergic neurons in the central nervous system [1–3]. This assertion resulted from studies which only considered the apparent inhibition of [^{3}H]DA uptake in synaptosomal preparation obtained from rat striata; however, DA releasing drugs would artefactually appear as uptake inhibitors [4, 5]. The data obtained in the preceding paper [6] with amitriptyline, butriptyline and iprindole were in agreement with such a view, since the DA release exactly coincided with the apparent DA uptake inhibition they produced. The aims of the present experiments were (i) to determine whether these effects were shared by other antidepressant drugs, (ii) to check whether other psychotropic agents were also active and (iii) to study the mechanisms involved in the releasing effect of antidepressants.

MATERIALS AND METHODS

The procedures for studying the DA release and/or DA uptake inhibition developed by drugs were similar to those described by Bonnet *et al.* [6] (this issue).

Crude synaptosomal preparations. The uptake and release were studied on crude synaptosomal preparations obtained according to Snyder *et al.* [7]. After centrifugation (1000 *g*, 10 min, 4°) of the striatal homogenates, the supernatant served as a source of crude synaptosomal preparations.

Uptake studies. Aliquots (50 μl) of synaptosomal preparation were preincubated for 5 min at 37° in 950 μl of Krebs–Ringer phosphate buffer containing nialamide (20 μM), 20×10^{-9} M [^{3}H]DA or [^{3}H]5HT were added for a 5 min incubation period. The reaction was stopped by dilution with ice-cold incubation medium and centrifugation (7000 *g*, 10 min, 4°). The pellet was rinsed and recentrifuged. Aliquots of the aqueous suspension of the final pellet served for the determination of radioactivity and protein concentration.

Double labelling test. Aliquots (400 μl) of synaptosomal preparation were preloaded with [^{14}C]DA (1.25 μM) similarly to the uptake studies previously described. The final pellet was resuspended in ice-cold medium; 100 μl aliquots of suspension of the preloaded preparation were added to 900 μl incubation medium maintained at 37° and containing [^{3}H]DA 10 nM, with or without the drug. Incubation was continued at 37° for 5 min and stopped by centrifugation (7000 *g*, 10 min, 4°). Aliquots of the supernatant served to determine the amount of released [^{14}C]DA. The final pellet was treated as previously described. The specific uptake of DA was defined as the difference between total uptake at 37° and non-specific uptake at 0°, and corrected for dilution of the [^{3}H]DA by the [^{14}C]DA released.

Release studies. Prelabelled synaptosomal suspensions were prepared as described in the double labelling study section, except that in the first incubation [^{3}H]DA or [^{3}H]5HT 1×10^{-7} M was added instead of [^{14}C]DA. The second incubation occurred without addition of labelled transmitter. The concentration–response curves of uptake inhibition and DA release were established from 3–8 experiments; in each 3–7 different concentrations were tested. The slopes of the curves were calculated by linear regression analysis (least squares method) of the effect's probits (uptake inhibition or release) vs log of drug concentrations for each experiment. IC_{50} (drug concentration inhibiting 50% of the control uptake) and RC_{50} (drug concentration releasing 50% of the [^{14}C]DA present in the preloaded preparation) were calculated by regression analysis of DA uptake or release percentages. Mean ± S.E.M. were compared by Student's *t*-test.

Effects of ions on the imipramine-induced release

of DA. When NaCl concentration was decreased the osmolarity was kept at 300 mosM with sucrose, and when KCl was 25 mM instead of 5 mM, the osmolarity was brought to 340 mosM either by the ionic concentration or by sucrose. Na_2EDTA 0.1 mM was included in experiments in which Ca^{2+} was deleted from the incubation medium.

Intrastriatal injections of imipramine. Rats anesthetized with chloral (300 mg/kg) were mounted in a stereotaxic frame. Coordinates of corpora striata ($A = 7.02$ mm, $L = 2.8$ mm, $H = 0.6$ mm) were chosen according to the atlas of König and Klippel [8]. Unilateral injections (8 μl) of imipramine 10^{-3} M (at pH 7.4 in incubation medium) were performed over 7 min, with a needle (0.4 mm diameter) connected to a microsyringe (Hamilton 10 μl); the contralateral striatum was injected with drug-free incubation medium.

Drugs. [^{3}H]DA HCl (6 Ci/mmole) was purchased from Amersham France and [^{14}C]DA acetate (53 mCi/mmole) from C.E.N. Saclay. Cocaine HCl was purchased from la Cooperative Pharmaceutique Française. The following drugs were generously donated by manufacturers: (+) and (−)amphetamine sulfate (Smith, Kline & French); pOH amphetamine HBr (Cassenne); nialamide, daledaline tosylate and doxepine HCl (Pfizer); nomifensine maleate (Hoechst); amitriptyline HCl and benztropine mesylate (Merck Sharp & Dohme-Chibret); butriptyline HCl (Auclair); iprindole HCl (Wyeth–Byla); pizotyline malate and dibenzepine HCl (Sandoz); promethazine HCl, chlorpromazine HCl and carpipramine diHCl (Specia); fenethazine HCl (Rhone Poulenc Santé); perlapine (Wander SA); doxylamine succinate (Merrell Toraude); dosulepine HCl (Boots–Dacour); viloxazine HCl (ICI Pharma France); benzoctamine, maprotiline, imipramine, desipramine, clomipramine and dibucaine, all as hydrochloride (Ciba–Geigy); medazepam (Roche); trihexyphenidyle HCl (Theraplix); flupentixol diHCl (Labaz); fluoxetine HCl (Lilly Research Center); methdilazine HCl (Allard). The initial dibucaine, perlapine and medazepam solutions (10^{-2} M) were prepared in HCl 0.1 N; all the other initial drug solutions were made in water. The subsequent dilutions were prepared with incubation medium: the initial solutions were diluted at least a hundred fold in the incubation medium.

RESULTS

Comparison between uptake inhibitory and releasing effects of drugs

Except for nomifensine, all antidepressant drugs tested on the double labelling test simultaneously displayed a releasing effect on [^{14}C]DA and an apparent inhibition of [^{3}H]DA uptake. For each drug, both effects occurred at very close concentrations; the IC_{50} and RC_{50} were well correlated ($R = 0.999$, $P < 0.001$) and in the range of 10^{-5} to 2.5×10^{-5} M except for dibenzepine (10^{-4} M) and viloxazine (5×10^{-4} M) (Fig. 1, Table 1). These IC_{50} values were about 10–100 times higher than those obtained for the uptake reference inhibitors nomifensine, amphetamine or cocaine. The slopes of the dose–response curves for antidepressants were also very similar and significantly correlated ($R = 0.789$, $P < 0.01$); in addition these slopes were clearly more steep than those of the reference inhibitors.

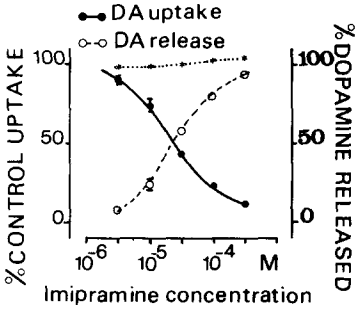

Fig. 1. Effects of imipramine on uptake and release of DA determined by the double labelling test. The synaptosomal preparation was preloaded with [^{14}C]DA (1.25 μM), rinsed, and then incubated at 37° for 5 min with [^{3}H]DA (10 nM) and various concentrations of imipramine. (○) DA release, (●) DA uptake corrected for dilution of [^{3}H]DA by released [^{14}C]DA, (*) inhibited uptake minus DA release. Each point is the mean ± S.E.M. of seven determinations.

The other tested drugs displayed the same potencies as the majority of antidepressants; their respective IC_{50} and RC_{50} values and the slopes of their dose–response curves were well correlated (respectively $R = 0.911$, $P < 0.001$ and $R = 0.829$, $P < 0.001$). Among the tested drugs, doxylamine demonstrated the lowest efficiency and chlorpromazine the highest; for dibucaine the slopes corresponding to the two phenomena were the most discordant: the slope of uptake inhibition curve was −1.79 whereas the slope of the release curve was 1.17.

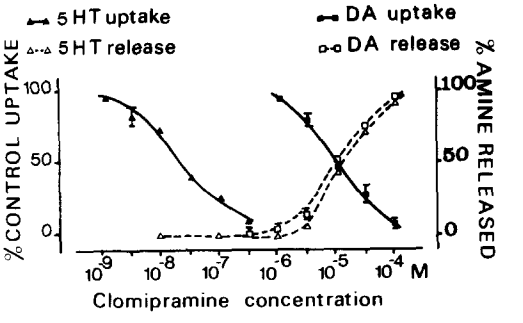

Fig. 2. Effects of clomipramine on uptake inhibition and release of 5HT and DA. The two batches of experiments were performed on the crude synaptosomal preparation obtained from striatum. For DA studies, the preparation was preloaded with [^{14}C]DA (1.25 μM), rinsed, and then incubated at 37° for 5 min with [^{3}H]DA (10 nM) and increasing concentrations of clomipramine. The 5HT studies determined the two phenomena separately: for 5HT release studies, the synaptosomal preparation was preloaded with [^{3}H]5HT (100 nM), rinsed, and then incubated at 37° for 5 min in the presence of increasing concentrations of clomipramine; for studies of [^{3}H]5HT uptake, the synaptosomal preparation was incubated at 37° for 5 min with [^{3}H]5HT (20 nM) in the presence of increasing concentrations of clomipramine. Each point is the mean ± S.E.M. of 3–5 determinations.

Table 1. Comparison of uptake inhibition and DA release elicited by antidepressants and miscellaneous agents. The synaptosomal preparation was preloaded with [^{14}C]DA (1.25 μM), rinsed, and then incubated at 37° for 5 min with [^{3}H]DA (10 nM) in the presence of increasing concentrations of drugs. The IC_{50} (μM), RC_{50} (μM) and the slope of the dose–response curves were determined by regression analysis. Three to eight different concentrations of each drug were tested and for each one, 3–7 experiments were performed.

Drug	IC_{50} (μM)	RC_{50} (μM)	Uptake inhibition slope	Dopamine release slope
Benztropine	0.24 ± 0.04	20	−1.31	1.5
Cocaine	1.41 ± 0.10	>100	−1.39	
d amphetamine	0.98 ± 0.08	300(a)	−1.19	0.67
l amphetamine	2.91 ± 0.65	300(a)	−0.94	0.60
pOH amphetamine	1.03 ± 0.20	10(a)	−0.97	0.75
Antidepressants				
Nomifensine	0.22 ± 0.03	>100	−1.34	
Clomipramine	10.2 ± 1.7	10.6 ± 0.9	−2.57	2.03
Maprotiline	10.9 ± 0.8	18.2 ± 1.7	−2.03	1.91
Carpipramine	12.8 ± 1.9	11.6 ± 1.6	−2.33	2.16
Pizotyline	17.4 ± 1.5	17.5 ± 2.0	−1.59	1.57
Amitriptyline	17.5 ± 4.6	20.1 ± 3.8	−1.85	1.85
Doxepine	20.3 ± 3.1	26.5 ± 2.9	−1.55	1.54
Dosulepine	22.1 ± 3.3	22.1 ± 1.3	−2.29	2.07
Desipramine	22.7 ± 2.7	37.8 ± 1.5	−1.96	2.10
Iprindole	22.9 ± 1.9	18.1 ± 1.2	−2.40	2.59
Butriptyline	25.5 ± 1.6	19.3 ± 0.5	−2.29	1.88
Imipramine	26.4 ± 2.5	26.4 ± 2.4	−1.81	2.07
Dibenzepine	160 ± 36	181 ± 21	−1.87	1.53
Viloxazine	505 ± 87	518 ± 48	−1.31	1.27
Miscellaneous				
Chlorpromazine	6.5 ± 0.9	11.6 ± 2.1	−2.19	1.91
Flupentixol	14.1 ± 0.2	15.1 ± 0.7	−2.60	2.23
Fluoxetine	14.7 ± 1.4	18.4 ± 2.1	−2.32	2.00
Methdilazine	15.5 ± 1.2	17.2 ± 1.0	−1.93	1.39
Promethazine	16.6 ± 2.2	21.6 ± 0.7	−1.66	1.57
Fenethazine	18.4 ± 5.0	21.8 ± 0.5	−1.69	1.67
Daledaline	21.8 ± 7.0	25.0 ± 6.0	−1.89	1.43
Trihexyphenidyl	22.0 ± 2.3	20.7 ± 0.8	−1.40	1.28
Medazepam	29.5 ± 8.7	32.9 ± 6.2	−2.11	1.75
Dibucaine	39.4 ± 8.0	15.7 ± 1.6	−1.79	1.17
Perlapine	83.7 ± 1.5	76.5 ± 15.6	−1.18	1.58
Benzoctamine	116 ± 3	132 ± 1.2	−2.78	2.14
Doxylamine	154 ± 68	367 ± 70	−1.05	1.06

(a) Approximate values in the lack of a 100% DA release.

Comparison of the relative potencies of clomipramine on the uptake inhibition and release of 5HT and DA

Clomipramine inhibited [^{3}H]5HT uptake with an IC_{50} of 2.7 ± 0.2 10^{-8} M. For 3 × 10^{-7} M this effect was complete and neither a 5HT or DA release nor an inhibition of DA uptake was observed. For higher concentrations, the drug released similarly both DA (RC_{50}: 10.6 ± 0.9 μM) and 5HT (RC_{50}: 14.3 ± 1.0 μM) and symmetrically inhibited the DA uptake (IC_{50}: 10.2 ± 1.7 μM) (Fig. 2).

Effects of modifications of the incubation medium on the DA release elicited by imipramine

K^+ concentration increase or Na^+ concentration decrease led to a significant increase of DA release. On the contrary, deleting Ca^{2+} from the incubation medium decreased the spontaneous DA release. However, in no case did these modifications affect the releasing effect of imipramine 3 × 10^{-5} M (Table 2). Lowering the medium temperature induced a decrease in the spontaneous DA release and an almost complete blockade of the induced DA release. Finally, neither nomifensine 10^{-6} M nor cocaine 10^{-5} M modified the DA release induced by imipramine.

Long-term effect of imipramine infusion on striatal DA uptake

The unilateral infusion of imipramine 10^{-3} M in the striatum did not modify, after a 7 days time lag, the DA uptake in this structure as compared to the contralateral side. The [^{3}H]DA uptake in the imipramine injected side was 103 ± 9% of the uptake in synaptosomes prepared from rat control, whereas in the sham injected side it was 104 ± 5% (N = 3–4).

Reversibility of the apparent DA uptake inhibition elicited by imipramine

When striatal synaptosomal preparations were exposed to a releasing concentration of imipramine

Table 2. Effects of cocaine, nomifensine and changes in incubation medium composition on the DA release induced by imipramine. The synaptosomal preparation was preloaded with [^{3}H]DA (100 nM), rinsed and then incubated at 37° for 5 min in reference incubation medium (drug-free, 134 mM NaCl, 4.5 mM KCl, 2.4 mM $CaCl_2$), in modified medium, with or without imipramine 3×10^{-5} M. Percentages of spontaneous DA release were determined in the imipramine-free incubation media; further DA release induced by imipramine was calculated as % of DA present in pellet at the end of the incubation in imipramine free medium according to [6]. Means ± S.E.M. were established from 3–7 experiments; *** different from respective reference medium at the level of $P < 0.001$; ** different from respective reference medium at the level of $P < 0.01$

Incubation condition	Spontaneous DA release	Further release of DA induced by imipramine
Incubation medium	42 ± 3%	78 ± 2%
Nomifensine (10^{-6} M)	53 ± 3%	81 ± 2%
Incubation medium	52 ± 1%	63 ± 1%
Cocaine (10^{-5} M)	55 ± 1%	66 ± 1%
Na^+ 134 mM	42 ± 1%	63 ± 3%
34 mM	60 ± 1%	57 ± 4%
K^+ 4.5 mM	47 ± 2%	52 ± 1%
25 mM	58 ± 1%***	53 ± 2%
Ca^{2+} 2.4 mM	44 ± 2%	57 ± 1%
0 mM + Edta 0.1 mM	33 ± 1%**	53 ± 2%
Incubation medium 37°	46 ± 1%	57 ± 3%
Incubation medium 0°	20 ± 2%***	−3 ± 1%***

(10^{-4} M) during 5 min, after two consecutive washes with drug-free medium (4 ml, 37°), there was a complete restoration of the [^{3}H]DA uptake which even reached 38.5 ± 2.5 pmole DA/mg protein as compared to 34.6 ± 1.4 pmole DA/mg protein for synaptosomes exposed to drug-free medium (mean ± S.E.M. of six experiments).

DISCUSSION

The dose–response curves for the apparent uptake inhibition and for the releasing effect established with antidepressants were strikingly symmetrical for each drug and the IC_{50} and RC_{50} values were similar. Therefore, if the DA efflux was subtracted from the DA influx, the net flux was null. Thus it seems that the uptake inhibition is only apparent, since the release induced by these drugs might account alone for the observed inhibition. One may ask whether the IC_{50} values which were determined may be reached during the treatment by these antidepressants and whether this effect participates in their therapeutic activity.

The pharmacokinetic studies of Jori *et al.* [9] and Nagy [10] indicated that imipramine, clomipramine and desipramine reached brain concentrations of about 10^{-6} to 10^{-5} M after different routes of administration. More recently Glotzbach *et al.* [11] determined that brain amitriptyline concentration, 15 min after an i.v. 1.75 mg/kg administration, was about 10^{-5} M. Halaris *et al.* [12] observed an early inhibition of *in vitro* DA uptake following a systemic administration (25 mg/kg) of clomipramine in rats. In the same field, Waldmeier [13] has shown that intraperitoneal administration of desipramine or doxepine (both 30 mg/kg) significantly decreased the brain DA concentration in rats. In spite of these data, it seems unlikely that the noted release might constitute the mechanism of the antidepressant activity since this effect is shared by miscellaneous drugs (neuroleptic, antihistaminic, antiparkinsonian, local anesthetic agents) and since the releasing effect elicited by these drugs does not seem specific of the neurotransmitter. This is especially supported by the effects of clomipramine on the uptake and release of 5HT and DA; this agent exerted its specific inhibition of 5HT uptake at concentrations about 1000 times lower than those inducing 5HT and DA release in a concordant manner. These results are in agreement with the reported effects of high concentrations of imipramine which inhibit *in vitro* the uptake of choline and amino acids [14–15] and which elicit an increased amino acid release *in vivo* [16].

Daniels *et al.* [17] and Langer *et al.* [18] reported that imipramine may be accumulated in synaptosomes. To elicit the DA release, imipramine might possibly have to enter the synaptosomes. However, this penetration does not involve the amine uptake systems since their blockade by cocaine, nomifensine or fluoxetine 10^{-5} M [19–21], or their functional inversion by low external Na^+ concentration [22] did not modify the imipramine-induced release of DA (data not shown for fluoxetine). The DA efflux would not involve the DA carrier since nomifensine did not modify the DA release elicited by imipramine whereas it antagonized the releasing effect of amphetamine. High K^+ concentrations are known to induce an exocytotic release of various neurotransmitters according to a strictly Ca^{2+}-dependent mechanism; however, Raiteri *et al.* [22] demon-

strated that this Ca^{2+} dependence is only relative for DA release. Since the Ca^{2+} deprivation appeared completely ineffective on the imipramine-induced release of DA in our experiments, one may suggest that this latter does not depend on exocytosis.

The releasing effect induced by the various drugs on the synaptosomal preparation does not seem to correspond to an irreversible lesion of dopaminergic endings since experiments in which unilateral slow infusion of imipramine in corpora striata or exposure of synaptosomal preparations to releasing concentration of imipramine did not modify the ensuing DA uptake.

Finally a direct interaction of imipramine with membrane components, possibly leading to a tensioactive effect, could be critical in the DA-induced release. This was suggested by the experiments of Ahtee [23], Seeman *et al.* [24] and Baur [25] which demonstrated a clear tensioactive effect of psychotropic agents.

In conclusion, it appears that various drugs in the range of 10^{-4} to 10^{-5} M exert an apparent inhibition of DA uptake which in fact corresponds to a DA release, as demonstrated by the double labelling test. The DA release elicited by these drugs is not specific to DA and most likely does not involve the DA carrier or exocytosis but probably implies a transient physico-chemical mechanism.

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