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# EFFECT OF NEUROLEPTICS AND OTHER DRUGS ON MONOAMINE UPTAKE BY MEMBRANES OF ADRENAL CHROMAFFIN GRANULES

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**1** The effects have been investigated of various reserpine-like, neuroleptic, antidepressant and other compounds on the adenosine-5'-triphosphate (ATP)-dependent uptake of noradrenaline (NA) (reserpine-sensitive) and tryptamine (reserpine-resistant) by membranes of isolated chromaffin granules of bovine adrenal medulla.

**2** Reserpine and Ro 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-hexahydro-11bH-benzo(a)quinolizine) as well as neuroleptics (e.g. chlorpromazine and haloperidol) inhibited the NA uptake, but the reserpine-like drugs were more potent. In contrast, Ro 4-1284 showed a considerably weaker effect than the neuroleptics in interfering with tryptamine uptake. Chlorpromazine had about the same potency in inhibiting NA and tryptamine uptake, whereas the action of haloperidol was more pronounced on the uptake of NA than of tryptamine.

**3** The relative potencies of neuroleptic drugs in inhibiting NA uptake by granule membranes *in vitro* corresponded only partly to their relative potencies in enhancing dopamine turnover *in vivo*.

**4** The inhibition of NA uptake by chlorpromazine and Ro 4-1284 appeared to be of the non-competitive type.

**5** Chlorpromazine did not influence the decrease in ATP induced by granule membranes in the incubation medium.

**6** Other basic, but not acidic compounds also inhibited NA uptake by granule membranes; their potency was of the order of that of chlorpromazine (antidepressants) or weaker (e.g. benzodiazepines).

**7** In conclusion, the mechanism of action of neuroleptics probably differs from that of reserpine-like drugs in the inhibition of monoamine uptake by membranes of catecholamine storage organelles. While interference with the granular storage of dopamine at the granule membrane level may contribute to the *in vivo* action of neuroleptics (e.g. in enhancing dopamine turnover), additional effects of these drugs must be involved *in vivo*, e.g. blockade of pre- and postsynaptic dopamine receptors.

## Introduction

Neuroleptic drugs of various chemical classes cause an increase of cerebral dopamine turnover (Pletscher & Kyburz, 1976). Several possible primary mechanisms leading to this functional change have been proposed. There is evidence for the presence of dopamine receptors at both pre- and postsynaptic sites, and inhibition of these receptors by the drugs probably leads to a feedback activation of neuronal dopamine turnover (Carlsson, 1975a). In addition, it has been suggested that neuroleptics affect presynaptic amine storage organelles (Seeman, 1972), although to date no direct proof for this hypothesis exists.

The present experiments were carried out in order to investigate the effects of neuroleptic and other drugs on the transport of labelled monoamines ([$^{14}$C]-noradrenaline and [$^{14}$C]-tryptamine) at the level of the membrane of amine storage organelles. The isolated membranes of adrenal chromaffin granules were used as a model system since it has not yet been possible to obtain pure amine storage organelles from the brain. Chromaffin granules are available in a highly purified form, and their membranes have been shown to take up biogenic amines by a specific, probably carrier-mediated adenosine-5'-triphosphate (ATP)-dependent transport mechanism (Taugner & Hasselbach, 1966; Taugner, 1971; Kirshner, 1974; Da Prada, Obrist & Pletscher, 1975). In the present investigations, [$^{14}$C]-noradrenaline ([$^{14}$C]-NA) was used instead of [$^{14}$C]-dopamine because the adrenal granule membranes contain dopamine-$\beta$-hydroxylase which transforms part of the [$^{14}$C]-dopamine into [$^{14}$C]-NA during the

uptake process, whereas [$^{14}$C]-NA is taken up unchanged (Helle, 1971; Da Prada *et al.*, 1975). Previous (Da Prada *et al.*, 1975) as well as preliminary experiments showed that [$^{14}$C]-NA and [$^{14}$C]-dopamine were taken up to about the same extent by the granule membranes and that neuroleptic drugs affected the uptake of both amines similarly.

## Methods

### *Preparation of membranes*

Chromaffin granules were isolated from bovine adrenal medulla, lysed by hypotonic shock, isolated by ultracentrifugation, washed, dialysed for 20 min and resuspended in Na-glycerophosphate buffer pH 7.4 as previously described (Da Prada *et al.*, 1975). The final suspension contained about 800 µg protein per ml.

### *Equilibrium dialysis*

Equilibrium dialysis experiments were carried out at 37°C using a 'Dianorm' equilibrium dialyser (Diachema AG, Birmensdorf/Zurich, Switzerland) made of Teflon and containing two microchambers ($M_1$ and $M_2$) separated by a 'spectrapor 2TM' membrane (molecular weight cut-off: 12,000–14,000) (Spectrum Medical Industries Inc., Los Angeles, U.S.A.). One chamber contained the granule membranes, while the radioactive amines and the drugs were added to both chambers in equal initial concentrations. The difference in counts between chamber $M_1$ (with membranes) and chamber $M_2$ (without membranes) at the end of the incubation period was taken as a measure of the amine uptake by the granule membranes and expressed in nmol per mg protein (for details see Da Prada *et al.*, 1975). According to previous findings, the majority of [$^{14}$C]-NA probably accumulates in the interior of the membrane vesicles (newly formed from membrane fragments), whereas only minor amounts are bound to the membranes (Agostini & Taugner, 1973).

### *Assay methods*

Aliquots (40 µl) of the incubation mixture of both microchambers were placed in 10 ml of scintillation mixture (Aquasol, New England Nuclear, Boston, U.S.A.) and counted in an Isokap/300 liquid scintillation system (Nuclear Chicago, U.S.A.). Protein determination was carried out by the colorimetric method of Lowry, Rosebrough, Farr & Randall (1951). The ATP content in the microchambers was measured by the luciferin-luciferase method (Holmsen, Holmsen & Bernhardsen, 1966) before and during incubation for 90 minutes.

### *Materials*

Adenosine-5′-triphosphate disodium salt, Sigma grade, was obtained from Sigma, St. Louis, Mo, U.S.A., (−)-noradrenaline-[carbinol-$^{14}$C]-(±)-bitartrate (54 mCi/mmol) and tryptamine [1-$^{14}$C]-bisuccinate (60 mCi/mmol) from New England Nuclear, Boston, U.S.A. The source of the non-labelled compounds examined as inhibitors of amine uptake was as follows: 2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-hexahydro-11bH-benzo(a)quinolizine (Ro 04-1284), chlordiazepoxide, chlorpromazine, diazepam, amitriptyline, chlorprothixene and methiotepin (F. Hoffmann-La Roche & Co. Ltd, Basel, Switzerland), imipramine, chlorimipramine and desmethylimipramine (Ciba-Geigy Ltd, Basel, Switzerland), thioridazine (Sandoz Ltd, Basel, Switzerland), clozapine (Wander Ltd, Bern, Switzerland), dextrobutaclamol and levobutaclamol (Ayerst Research Laboratories, Montreal, Canada), reserpine and phenobarbitone (Siegfried Ltd, Zofingen, Switzerland), γ-hydroxybutyrate (Aldrich Chemical Comp. Inc., Milwaukee, Wisc., U.S.A.), γ-aminobutyric acid (Serva Feinbiochemica, Basel, Switzerland), haloperidol and pimozide (Janssen, Beerse, Belgium), azure A (Aldrich, Beerse, Belgium) and fluorescein (Merck, Darmstadt, Germany).

## Results

### *Neuroleptics and reserpine-like drugs*

Chlorpromazine, haloperidol and the reserpine-like compound Ro 04-1284 (Pletscher, Brossi & Gey, 1962) inhibited the ATP-dependent uptake of NA by chromaffin granule membranes. The inhibition increased with increasing concentrations of the drugs and appeared to be of the non-competitive type (Figures 1 and 2). Various other neuroleptic drugs as well as reserpine decreased the NA uptake. Reserpine and Ro 04-1284 were between 30 and 2000 times more potent than the neuroleptics. Among the latter, haloperidol was the most potent compound exhibiting an $EC_{50}$ of $5 \times 10^{-6}$ M while the least potent drug, clozapine, differed by more than an order of magnitude. The $EC_{50}$ of L-butaclamol was only twice as high as that of D-butaclamol (Table 1).

### *Antidepressants*

These drugs also caused a concentration-dependent inhibition of the NA uptake by granule membranes. The compounds did not vary markedly in their potency which was of the same order as that of chlorpromazine and thioridazine, and thus lower than that of haloperidol (Table 1).

*Miscellaneous compounds*

Other basic substances, e.g. benzodiazepines and the colourant Azure A, also interfered with the NA uptake in a concentration-dependent manner. Their potency, however, was rather weak, i.e. in the order of or below that of clozapine. In contrast, with the acidic compounds γ-hydroxybutyric acid, fluorescein, phenobarbitone as well as with γ-aminobutyric acid (GABA) little inhibition of NA uptake by granule membranes was detectable (Table 1).

*Reserpine-resistant uptake*

In contrast to reserpine which did not inhibit the uptake of tryptamine (Da Prada *et al.*, 1975), chlorpromazine and haloperidol interfered with the uptake of this amine in a concentration-dependent manner. The $EC_{50}$ of chlorpromazine for inhibition of [$^{14}$C]-tryptamine uptake (about $4 \times 10^{-5}$ M) was similar to that for inhibition of [$^{14}$C]-NA uptake ($4 \times 10^{-5}$ M), whereas haloperidol was less potent in inhibiting tryptamine than NA uptake ($EC_{50}$ of haloperidol $7 \times 10^{-5}$ M and $5 \times 10^{-6}$ M, respectively). The reserpine-like compound Ro 04-1284 also interfered with tryptamine uptake, but its $EC_{50}$ ($8 \times 10^{-4}$ M) was considerably higher than that for inhibition of NA uptake ($1.5 \times 10^{-7}$ M) (Figure 3).

**Table 1** Molar concentration of various compounds causing a 50% inhibition of the (–)-[$^{14}$C]-noradrenaline uptake ($EC_{50}$) by membranes of adrenal chromaffin granules.

| *Drug* | $EC_{50}$ |
|---|---|
| *Reserpine-like* | |
| Reserpine | $4 \times 10^{-8}$ |
| Ro 04-1284 | $1.5 \times 10^{-7}$ |
| *Neuroleptics* | |
| Haloperidol | $5 \times 10^{-8}$ |
| Pimozide | $1 \times 10^{-5}$ |
| D-Butaclamol | $2 \times 10^{-5}$ |
| Chlorprothixene | $3 \times 10^{-5}$ |
| Thioridazine | $3 \times 10^{-5}$ |
| Chlorpromazine | $4 \times 10^{-5}$ |
| L-Butaclamol | $4 \times 10^{-5}$ |
| Methiotepin | $5 \times 10^{-5}$ |
| Clozapine | $7 \times 10^{-5}$ |
| *Antidepressants* | |
| Chlorimipramine | $3 \times 10^{-5}$ |
| Imipramine | $4 \times 10^{-5}$ |
| Desimipramine | $4 \times 10^{-5}$ |
| Amitriptyline | $4 \times 10^{-5}$ |
| *Miscellaneous* | |
| Diazepam | $7 \times 10^{-5}$ |
| Azure A | $9 \times 10^{-5}$ |
| Chlordiazepoxide | $5 \times 10^{-4}$ |
| Phenobarbitone | $>5 \times 10^{-4}$ |
| γ-Aminobutyric acid | $>3 \times 10^{-3}$ |
| Fluorescein | $>5 \times 10^{-4}$ |
| γ-Hydroxybutyrate | $>3 \times 10^{-3}$ |

Incubation was at 37°C for 30 min in the presence of ATP. Initial concentrations of ATP and [$^{14}$C]-NA were 5 mM and 50 μM respectively. The values were determined graphically from concentration-inhibition curves (see Figures 1 and 3).

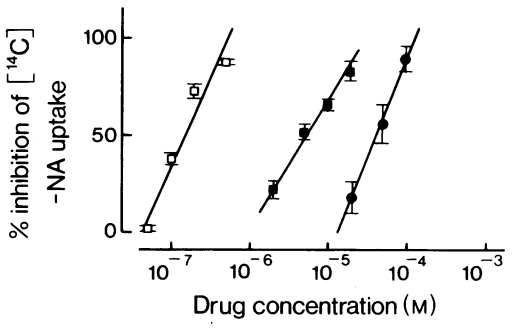


**Figure 1** Inhibition of the uptake of (–)-[$^{14}$C]-noradrenaline ([$^{14}$C]-NA) in membranes of adrenal chromaffin granules by Ro 04-1284 (□), chlorpromazine (●) and haloperidol (■). Incubation at 37°C for 30 min in the presence of ATP. Initial concentrations of ATP and [$^{14}$C]-NA were 5 mM and 50 μM respectively. Each point is an average of 3 experiments. Vertical lines show s.e. mean.

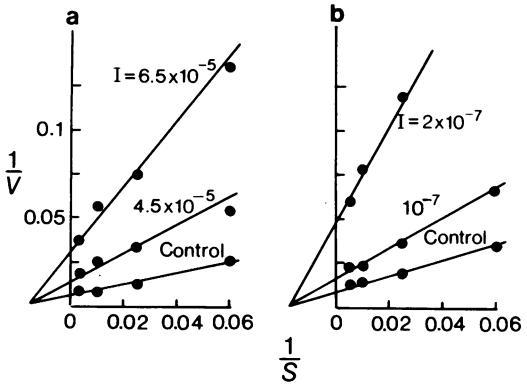


**Figure 2** Double reciprocal plot (Lineweaver-Burk) of the effect of (a) chlorpromazine and (b) Ro 04-1284 on the uptake of (–)-[$^{14}$C]-noradrenaline ([$^{14}$C]-NA) by membranes of adrenal chromaffin granules incubated for 30 min at 37°C in the presence of ATP (initial concentration 5 mM). $V$=initial velocity of uptake (nmol per mg of protein/15 min); $S$=μmolar concentration of [$^{14}$C]-NA. I=molar concentration of drugs. Typical experiments.

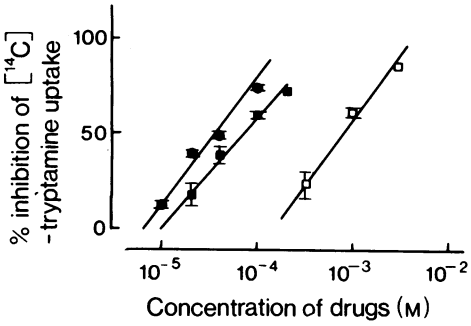


**Figure 3** Inhibition of the uptake of [$^{14}$C]-tryptamine by membranes of adrenal chromaffin granules caused by various concentrations of Ro 04-1284 (□), chlorpromazine (●) and haloperidol (■). Incubation at 37°C for 30 min in the presence of ATP. Initial concentrations of ATP and [$^{14}$C]-tryptamine were 5 mM and 50 μM respectively. Each point is an average of 3 experiments. Vertical lines show s.e. mean.

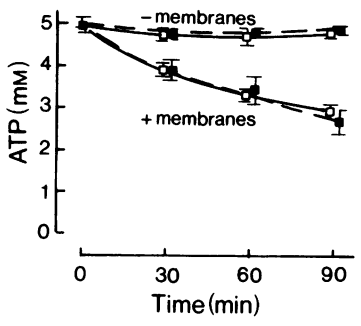


**Figure 4** Effect of chlorpromazine on the concentration of adenosine-5′-triphosphate (ATP) in the medium during incubation at 37°C with or without membranes of chromaffin granules. Each point is an average of 4 measurements performed in 2 experiments. Vertical lines show s.e. mean. Solid line: no chlorpromazine; broken line: with $10^{-4}$ M chlorpromazine.

*ATP degradation*

Figure 4 shows that in the absence of membranes the ATP content of the medium remained unaltered for at least 90 minutes. However, in the presence of chromaffin granule membranes in one chamber there was a progressive decrease of the ATP content in both chambers and, as reported previously (Da Prada *et al.*, 1975), an appearance of adenosine-5′-diphosphate and adenosine-5′-monophosphate. Chlorpromazine ($10^{-4}$M) did not influence the ATP decrease induced by granule membranes (Figure 4).

## Discussion

The present results demonstrate that not only reserpine-like drugs (Taugner & Hasselbach, 1966; Da Prada *et al.*, 1975) but also neuroleptics inhibit the amine uptake by membranes of amine storage organelles. However, the mechanism of action of these two classes of drugs seems to differ from that of false neurotransmitters like octopamine. In fact, the antagonism between NA and octopamine was competitive (Da Prada *et al.*, 1975), while that between NA and Ro 04-1284 or chlorpromazine appeared to be of the non-competitive type. On the other hand, there was also a difference between neuroleptics and reserpine-like drugs. Neuroleptics, in addition to inhibiting the reserpine-sensitive NA uptake, also showed a relatively strong interference with the reserpine-resistant tryptamine uptake (compared to their effect on NA uptake), whereas reserpine-like drugs had a much greater effect on the reserpine-sensitive uptake (see Figures 1 and 3 and Da Prada *et al.*, 1975). These findings are compatible with the view that neuroleptics, such as chlorpromazine, cause an alteration of the physicochemical properties (e.g. fluidization) of artificial as well as biological membranes (e.g. of erythrocytes) (Seeman, 1972). This probably leads to relatively unspecific impairment of the monoamine transport system, whereas the more potent reserpine-like drugs might act through a more specific mechanism. It does not seem that chlorpromazine exerts its action by inhibiting membrane ATP-ase, since the drug, in concentrations causing a virtually complete block of monoamine uptake, did not greatly influence the ATP decrease in the medium induced by granule membranes. On the other hand, it has been suggested that reserpine interferes specifically with a transport ATP-ase (Taugner & Hasselbach, 1966), but no proof for this hypothesis exists as yet.

There might be some differences in the mode of action of chlorpromazine and haloperidol, since chlorpromazine affected NA and tryptamine uptake to the same extent, while haloperidol was more potent regarding interference with NA uptake.

The question arises whether the inhibition of amine uptake by reserpine-like and neuroleptic drugs seen in granule membranes *in vitro* is relevant for the *in vivo* action of the drugs. Both types of compounds enhance the dopamine turnover in brain *in vivo*.

Reserpine is thought to impair primarily the storage of amines in the presynaptic storage organelles (Carlsson, 1975b) leading to a virtually complete depletion of their dopamine content. The potent inhibitory effect of reserpine and Ro 4-1284 in the present as well as in previous experiments (Taugner & Hasselbach, 1966; Da Prada *et al.*, 1975) provides

direct evidence that reserpine-like drugs exert their action at the level of the membrane of the storage organelles. As a consequence of the depletion of presynaptic dopamine, the neuronal turnover of the amine is enhanced, be it by removal of end-product inhibition of intraneuronal tyrosine hydroxylase or by disinhibition of pre- and postsynaptic dopamine receptors.

With regard to neuroleptics the relevance of the *in vitro* effect found in these experiments for their *in vivo* action is not fully clear. The action of these drugs on granule membranes was less potent than that of reserpine-like compounds. Furthermore, according to previous findings, the dopamine-sensitive adenylate cyclase in striatal homogenates (Iversen, 1975) which is considered to be related to the dopamine receptor (Kebabian, Petzold & Greengard, 1972), the electrically stimulated dopamine release from striatal slices (Seeman & Lee, 1975; Iversen, Horn & Miller, 1975), the presynaptic action of apomorphine on dopamine formation in striatal synaptosomes (Iversen, Rogawski & Miller, 1976) and the binding of haloperidol to postsynaptic receptors (Creese, Burt & Snyder, 1976) showed greater sensitivity to neuroleptics (one, to more than two, orders of magnitude) than the NA uptake by adrenal granule membranes. These findings might argue against neuroleptics exerting an effect *in vivo* by interfering with granular amine storage, but this argument is not fully convincing. Isolated membranes of adrenal chromaffin granules may be less sensitive to neuroleptics than brain monoamine storage organelles *in situ*, in fact, in brain slices the effective concentration of reserpine (which acts primarily on monoamine storage organelles) in enhancing spontaneous release of [$^{14}$C]-dopamine was of the order of $10^{-9}$ M (Seeman & Lee, 1974), whereas in the present experiments this order was $10^{-8}$ M. Also, the daily dose of neuroleptics, especially in psychiatry, is higher than that of reserpine (e.g. 200 mg chlorpromazine or approximately $10^{-5}$ M/kg) and furthermore neuroleptics are administered for a prolonged period of time, whereas the present work deals with acute experiments. An enhanced release of dopamine (which might be due to an impairment of amine storage) has, for instance, been suggested as the cause of some side effects of neuroleptic drugs, e.g. the tardive dyskinesia (Seeman & Lee, 1974).

An action of neuroleptics on granular amine storage may also contribute to an increased turnover of dopamine *in vivo*, although neuroleptics, in contrast to reserpine-like drugs, do not cause a depletion of presynaptic dopamine stores (probably owing to a compensatory increase of dopamine synthesis). It has been suggested that an increased release of dopamine due to the action of neuroleptics on dopamine stores removes end-product inhibition of tyrosine hydroxylase, thereby enhancing dopamine synthesis (Seeman, 1972).

While an action of neuroleptic drugs on the membranes of dopamine storage organelles may be a contributory element, other factors must be involved in the *in vivo* action of neuroleptic drugs. Thus, D-butaclamol enhanced the turnover of dopamine *in vivo* much more than the L-enantiomer (which was virtually inactive) (Lippmann, Puglsey & Merker, 1975), whereas in the NA uptake experiments with granule membranes L-butaclamol was only slightly inferior to the D-form. Furthermore, clozapine *in vivo* was at least one order of magnitude less potent than chlorpromazine (Pletscher, 1975), whereas *in vitro* the difference was smaller. Finally, antidepressant drugs and benzodiazepines which differ from neuroleptics in their clinical spectrum as well as in their effect on cerebral dopamine turnover (Da Prada & Pletscher, 1966; Bartholini, Keller, Pieri & Pletscher, 1973), also showed some inhibitory effect on NA transport in granule membranes. These findings are probably due to the fact that additional mechanisms are involved in the *in vivo* action of the various neuroleptics, e.g. stimulation of pre- and postsynaptic dopamine receptors, inhibition of $Ca^{2+}$ influx in presynaptic nerve terminals leading to a blockade between nerve impulse and neurosecretion (Seeman & Lee, 1975). Furthermore, the action of drugs on granular membranes may be masked by an interference of the drugs with other processes, e.g. amine uptake at the neuronal membrane (antidepressants (Halaris, Belendiuk & Freedman, 1975)) or with other transmitters, e.g. GABA (benzodiazepines (Keller, Schaffner & Haefely, 1976)). Finally, *in situ*, the various neuroleptics and other drugs are possibly transported across the neuronal membrane to different degrees leading to differences in the intraneuronal drug concentration.

It is of interest that different types of basic substances interfered with the amine transport in granule membranes, whereas acidic compounds did not cause a relevant inhibition of the NA uptake (Table 1). These results are in agreement with previous findings in blood platelets where various basic compounds, especially neuroleptics, reserpine-like substances and colourants (but according to preliminary results not acidic dyes) showed a rather selective localization at the level of the 5-hydroxytryptamine storage organelles (Da Prada & Pletscher, 1969; 1975).

Some preliminary results of this work were presented at the Symposium on Physiology and Pathology of Biological Membrane Functions organized by the Swiss Academy of Medical Sciences in the spring of 1976.

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