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# TRANSPORT OF MONOAMINES IN MEMBRANES OF ADRENAL CHROMAFFIN GRANULES: PHYSIOLOGICAL AND PHARMACOLOGICAL ASPECTS

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## 1. Introduction

Release of monoamines, e.g. catecholamines and 5-hydroxytryptamine (5HT) from presynaptic nerve terminals as well as their re-uptake into these terminals are essential steps in the process of neurohumoral transmission in monoaminergic neuronal systems. The bulk of the amines is located in specific storage granules and liberated from these during neuronal activity (KOPIN I.J. 1966). Two subcellular structures are important for the re-uptake process of the amines, i.e. the neuronal membrane and the amine storage organelles. Good evidence exists for an active uptake mechanism at the level of the neuronal membrane which is dependent upon $Na^+$, $K^+$-stimulated ATPase and inhibited by ouabaine and various other drugs. In the storage organelles, two mechanisms seem to be of importance, i.e. an intragranular intermolecular interaction between the amines, adenosine-5'-triphosphate (ATP), bivalent metals and possibly proteins, and transport through the granule membrane (PLETSCHER et al. 1974, KOPIN 1966, DA PRADA et al. 1975). No detailed investigations on these granular mechanisms in the central nervous system exist, since the isolation of pure amine storage organelles from this tissue has not yet been achieved. Therefore, other amine storage organelles, e.g. adrenal chromaffin granules, noradrenaline (NA) storing granules of peripheral (e.g. splenic) nerves and 5HT organelles of blood platelets are taken as models to study granular amine storage.

The present work deals with some physiological aspects of monoamine uptake and its pharmacological modification in isolated membranes of bovine adrenal chromaffin granules.

## 2. Experimental

Chromaffin granules from bovine adrenal medulla were isolated in highly purified form and submitted to osmotic shock as described earlier (DA PRADA et al. 1975) in order to get rid of the majority of the granule contents (e.g. catecholamines and ATP) (Table 1). After resuspension in isotonic buffer, the granule membranes which had reclosed to form vesicular structures, as shown by electron microscopy, were incubated in a microdialyzer with radioactive amines in the absence or presence of drugs. The microdialyzer consisted of two equal microchambers separated by a semipermeable membrane (molecular weight cut-off 12,000 - 14,000), whereby one chamber ($M_1$) contained the granule membranes (Fig. 1). The initial concentration of amines and drugs in both chambers was equal. The difference in the 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 for the amine taken up by the granule membranes and expressed in nmoles per mg protein (determined colorimetrically). The determination of endogenous catecholamines of the membranes of chromaffin granules was carried out spectrophotofluorimetrically, the assay of ATP with luciferine-luciferase (DA PRADA et al. 1975).

## 3. Amine uptake

Numerous investigations have shown that isolated membranes of adrenal chromaffin granules take up NA and other biogenic amines from the incubation medium. This is a net uptake, not an exchange with endogenous catecholamines of the membranes, since the endogenous catecholamines did not decrease in the course of the incubation (DA PRADA et al. 1975). According to previous results, the majority of the exogenous amines seems to accumulate in the interior of the membrane vesicles (AGOSTINI et al. 1973).

The major part of the amines enters the granule membranes by an uptake process which shows the following characteristics (DA PRADA et al. 1975, PLETSCHER et al. 1973):

3.1. The uptake occurs against a considerable concentration gradient as, for instance, the $^{14}$C-NA concentration in the granule membranes may rise to more than 100 times that in the incubation medium.

3.2. The amine uptake in relation to the amine concentration in the medium follows saturation kinetics (Fig. 2).

3.3. There is competitive antagonism between various monoamines (e.g. NA and octopamine) regarding their uptake.

3.4. The amine uptake depends on the presence of ATP in the medium. Without the nucleotide, only little amine is taken up (Fig. 2, Table 2).

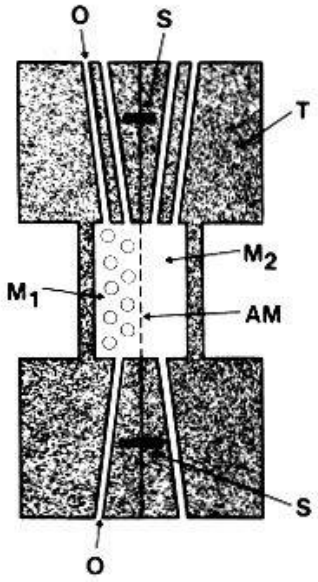


Fig. 1. Model of microdialyzer. AM = semipermeable artificial membrane; $M_1$ = microchamber (200 µl) containing biological membranes; $M_2$ = microchamber (200 µl) containing incubation medium only; O = open outlet (plugged during incubation) for filling or removal of incubation mixture; T = Teflon. After filling, five dialyzers were fixed on a rotor and submerged in a water bath in order to maintain a constant temperature.

Table 1. Content of Catecholamines and ATP in nmols/mg protein of whole chromaffine granules and their membranes.

| Constituents | Whole granules | Granule membranes |
|---|---|---|
| Catecholamines | 2521 | 106 |
| ATP | 596 | 5 |

3.5. There is a marked temperature dependence with regard to the amine uptake. At 25°C the uptake is decreased compared to 37°C and at 2°C at the best small amounts of amines are taken up.

3.6. N-Ethylmaleimide, an inhibitor of $Mg^{++}$-stimulated ATPase, but not ouabaine, causes marked inhibition of amine uptake.

3.7. A stoichiometric relation exists between ATPase activity of the membranes and amine influx through the membranes.

3.8. The membranes discriminate between various amines with regard to the magnitude of uptake (Table 2).

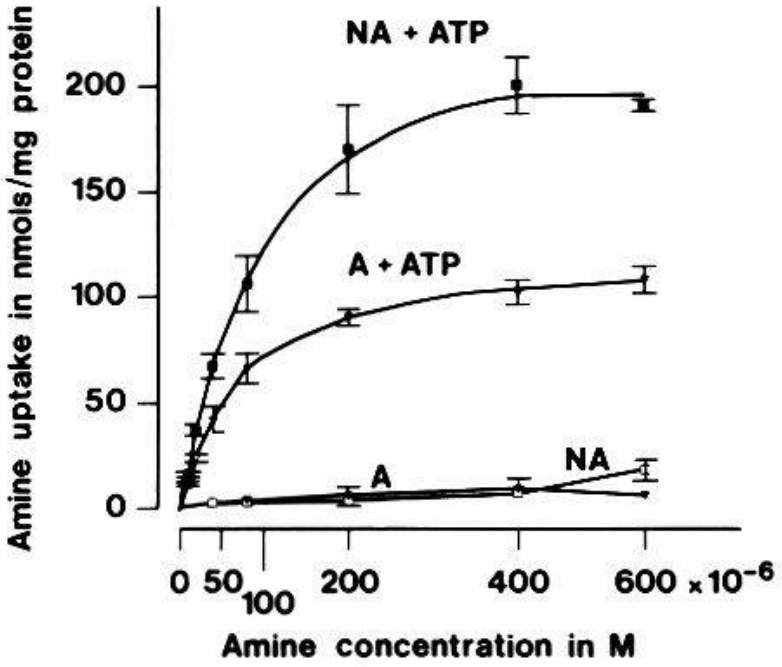


Fig. 2. Uptake of labelled (-)-noradrenaline (NA) and (±)-adrenaline (Ad), by isolated membranes of bovine adrenal chromaffin granules incubated at 37°C for 30 min. in $MgCl_2$-containing medium with various concentrations of the amines in the presence and absence of adenosine-5'-triphosphate (ATP, initial concentration 5 mM). The points are means of 3 - 4 experiments. Vertical bars show s.e. mean. (■)NA + ATP; (□) NA; (▽) Ad + ATP; (▼) Ad. (2)

Table 2. Uptake of various radioactive amines by isolated membranes of bovine adrenal chromaffin granules incubated in a $MgCl_2$-containing medium at 37°C for 30 minutes.

| Incubation in | Presence of ATP | Absence of ATP |
|---|---|---|
| Dopamine | 80.09 ± 7.26 | 2.49 ± 0.32 |
| (-)-Noradrenaline | 76.73 ± 5.56 | 4.30 ± 0.86 |
| 5-Hydroxytryptamine | 74.04 ± 7.20 | 4.02 ± 1.23 |
| (±)-Adrenaline | 64.64 ± 6.77 | 2.21 ± 0.57 |
| (±)-Octopamine | 54.02 ± 3.39 | 1.38 ± 0.47 |
| Tyramine | 37.93 ± 3.08 | 1.62 ± 0.03 |
| (±)-Metaraminol | 28.86 ± 0.93 | 0.65 ± 0.27 |
| Trytamine | 22.47 ± 0.77 | 10.52 ± 1.23 |
| Phenylethylamine | 14.28 ± 0.85 | 2.20 ± 0.58 |
| Histamine | 5.32 ± 0.40 | 1.68 ± 0.44 |

Initial concentrations in incubation medium: amines 45 μM, adenosine-5'-triphosphat (ATP) 5 mM. The figures represent means with s.e. mean of 3 - 6 experiments and are indicated in nmol per mg membrane protein. (2)

3.9. The ATP-stimulated uptake of NA shows some stereospecificity since (-)NA is taken up to a larger extent than (+)NA.

From these and other findings it can be concluded that the transport of amines at the level of the granule membrane does not occur by mere diffusion, but mainly by a specific mechanism, probably a carrier transport, which may depend on metabolic energy. Thereby $Mg^{++}$-stimulated ATPase which occurs in the chromaffin granule membrane seems to play a role. The above mentioned difference in uptake of the various amines may be due to differences in their stereochemical configuration with regard to that of the carrier.

The ability of the membranes to take up various amines and the competitive antagonism of the amines are of pharmacological interest. It probably explains why an amine not normally occurring in the granules may accumulate in these if it is present in increased amounts (e.g. due to increased formation or decreased metabolism). Interestingly, octopamine, which in the presence of ATP accumulates in the chromaffin granule membranes, has been claimed to accumulate intraneuronally (probably in the storage organelles) after inhibition of monoamine oxidase and was postulated to function as a false neurotransmitter (KOPIN et al. 1965).

## 4. Pharmacological investigations

### 4.1. Reserpine-like compounds

In animals or isolated tissues reserpine and similar compounds (e.g. benzoquinolizine (a) derivatives (PLETSCHER et al. 1962)) inhibit the storage of several monoamines (e.g. catecholamines and 5HT) in various tissues. This inhibition is thought to occur at the level of the monoamine storage organelles; the exact site of action of reserpine has, however, not been elucidated in these experiments. On the other hand, the storage of some amines (e.g. metaraminol) is only partly inhibited by reserpine so that a reserpine insensitive mechanism of amine uptake has been postulated (DA PRADA et al. 1975).

Experiments regarding the effect of reserpine-like drugs on the uptake of monoamines by isolated membranes of chromaffin granules have brought some clarification although many questions still remain unsolved. Thus, both reserpine (Fig. 3) and a benzoquinolizine derivative with reserpine-like action (Ro 04-1284*) (PLETSCHER et al. 1962) caused virtually complete inhibition of $^{14}C$-NA uptake, the $ED_{50}$ of the two drugs being of the order of $10^{-8}$ and $10^{-7}$ M respectively. As shown for Ro 04-1284, the inhibition was of the non-competitive type. These experiments therefore indicate that reserpine and similar compounds probably

*) 2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,5,7,-hexahydro-11bH-benzo (a) quinolizine

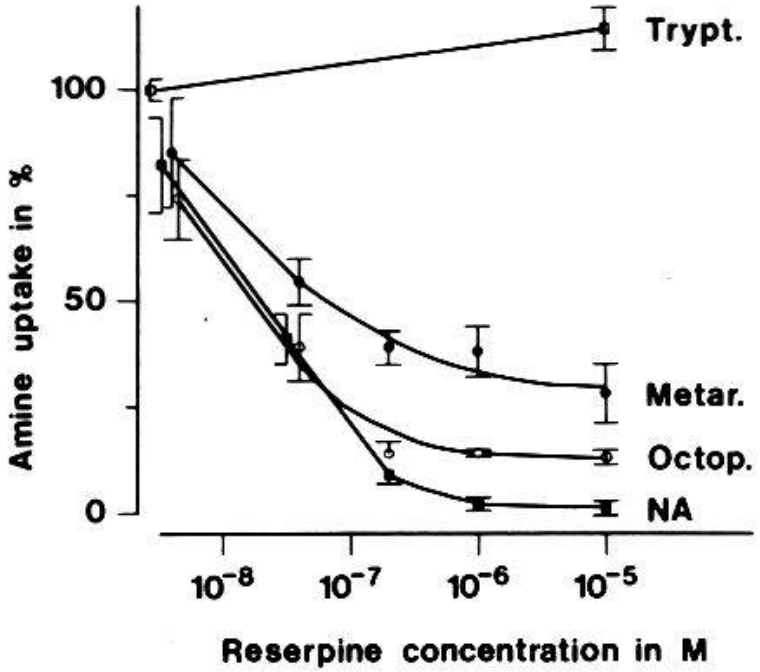


Fig. 3. Effect of various concentrations of reserpine on the uptake of labelled (-)-noradrenaline (■), (±)-octopamine (o), (±)-metaraminol (●) and tryptamine (□) at 37°C by isolated chromaffin granule membranes incubated for 30 min. at 37°C in $MgCl_2$-containing medium in the presence of ATP. Initial concentrations: labelled amines 45 μM, ATP 5 mM. The points are means of 4 - 6 experiments and are expressed as a percentage of the values obtained after incubation of membranes in the same media but without reserpine (controls, uptake = 100 %). Vertical bars show s.e. mean.

Table 3. $ED_{50}$ means concentration of drug causing 50 % inhibition of noradrenaline (NA) uptake.

| Neuroleptic | $ED_{50}$ (M) |
|---|---|
| Clozapine | $7.10^{-5}$ |
| Methiothepine | $5.10^{-5}$ |
| Chlorpromazine | $4.10^{-5}$ |
| Thioridazine | $3.10^{-5}$ |
| Pimozide | $10^{-5}$ |
| Haloperidol | $6.10^{-6}$ |

exert their action at the level of the granule membrane. The mechanism of action of the drug, however, is unknown. Reserpine does not cause a major inhibition of the total ATPase activity of the membranes, but a minor effect cannot be excluded. It has therefore been postulated that the drug might interfere with a specific transport ATPase (TAUGNER et al.

1966), which possibly exists in the granule membrane together with one or more reserpine-resistant ATPases (PLETSCHER et al. 1973).

The uptake by the granule membranes of amines like tryptamine, metaraminol, tyramine and octopamine showed no or only partial inhibition by reserpine (Fig. 3). The reserpine-insensitive part of the uptake was, however, at least partly dependent on ATP, since for the four amines mentioned the uptake under the influence of reserpine was more marked in the presence than in the absence of ATP (DA PRADA et al. 1975).

These findings thus directly demonstrate the existence at the granule membrane level of a reserpine-resistant amine uptake. Therefore the reserpine-resistant metaraminol uptake postulated on the basis of earlier experiments is probably located in the granule membrane.

## 4.2. Neuroleptics

Neuroleptics similarly to reserpine-like drugs enhance the dopamine (DA) turnover in dopaminergic neurons. Whereas the primary site of action of reserpine-like drugs is thought to be the presynaptic DA storage organelle, no clarity exists regarding the primary effect of neuroleptics which leads to the enhancement of DA turnover. Presynaptic mechanisms (blockade of presynaptic coupling between impulse and neurosecretion (SEEMAN et al. 1975), increase of spontaneous DA release (SEEMAN et al. 1974), blockade of presynaptic DA receptors (KEHR et al. 1972)) as well as postsynaptic mechanisms (blockade of postsynaptic DA-receptors (CARLSSON et al. 1963)) are being discussed. Therefore investigations were carried out to ascertain whether neuroleptics had a direct effect on the transport of monoamines by chromaffin granule membranes.

Various neuroleptics caused a dose-dependent inhibition of the $^{14}$C-NA uptake by the chromaffin granule membranes whereby the $ED_{50}$ of the compounds was different (Table 3). According to preliminary results, the inhibition appears to be of the non-competitive type. It is of interest that those compounds with the most potent enhancing action on neuronal DA turnover in vivo (i.e. pimozide and haloperidol) were also most potent in inhibiting $^{14}$C-NA uptake. However, on the other hand, only a minor stereospecific difference in the inhibitory effect of D- and L-butaclamol in vitro could be observed, although the D-isomer shows much higher potency in enhancing neuronal DA turnover in vivo (LIPPMANN et al. 1975).

It was of interest to investigate whether neuroleptics also interfered with the reserpine-resistant amine uptake. Experiments with tryptamine whose uptake is entirely reserpine-resistant showed that, in the presence of ATP, chlorpromazine inhibited the uptake of tryptamine to a similar degree to that of NA ($ED_{50}$ about $4.10^{-5}$M in both cases). Therefore, the mechanisms of action by which reserpine and chlorpromazine interfere with the amine trans-

port at the granule membrane level seem to be different: The action of chlorpromazine is probably less specific than that of reserpine.

It may be speculated that neuroleptics exert an effect on various types of subcellular membranes in monoaminergic systems resulting in several consequences e.g. interference with granular amine storage (granule membrane), blockade of presynaptic coupling between nerve impulse and neurosecretion (neuronal membrane), blockade of DA receptors (pre- and postsynaptic membrane). Several investigators have indeed shown that neuroleptics such as chlorpromazine cause changes in the physicochemical properties (e.g. fluidization) of artificial as well as biological membranes (e.g. of erythrocytes) (SEEMAN 1972).

Based on these results it is conceivable that neuroleptics and reserpine-like drugs have a common site of action, i.e. the membrane of the amine storage organelles, although the mechanisms by which the two types of drugs interfere with this membrane are different. The action of the neuroleptics on the granule membrane, as that of reserpine-like drugs, might be involved in the enhancement of the neuronal DA turnover. However, additional factors must determine the neuroleptic induced enhancement, e.g. the permeability of the neuronal membrane for the drugs (possibly explaining the stereo-selective effect of D-butaclamol in vivo), a change in the physiological properties of this membrane (leading to a blockade of coupling between impulse and DA release), a blockade of specific DA receptors.

## 5. Summary and conclusion

5.1. The membranes of isolated bovine adrenal chromaffin granules take up catecholamines by an ATP-dependent process which fulfills many criteria of a carriermediated energy-requiring transport. This transport together with an intragranular mechanism probably explains the high storage capacity of the chromaffin granules for catecholamines.

5.2. Other amines (e.g. 5-hydroxytryptamine (5HT), octopamine) are also taken up by the membranes, but to a different degree. This probably explains why amines normally not present in storage organelles, if available in increased amounts, may accumulate within these organelles and function as false neurohumoral transmitters.

5.3. Two mechanisms of amine uptake seem to exist at the level of the granule membranes, one reserpine-sensitive (transporting, for instance, catecholamines and 5HT), and one reserpine-resistant (transporting tryptamine and partly metaraminol). Both mechanisms depend on ATP.

5.4. Chlorpromazine interferes with the reserpine-sensitive as well as the reserpine-resistant

uptake of noradrenaline (NA) by granule membranes to a similar degree. Consequently the mechanisms of action of neuroleptics and reserpine at the granule membrane level are probably different.

5.5. There is only an incomplete parallelism between the inhibition of NA-uptake by granule membranes in vitro and the degree of enhancement of neuronal DA turnover in vivo caused by various neuroleptics. Therefore, factors other than interference with the amine transport through the granule membranes must be co-determinant in the neuroleptic induced enhancement of DA turnover in vivo.

## Schlussfolgerungen und Zusammenfassung

1. Die Membranen von isolierten chromaffinen Granula des Rinder-Nebennierenmarkes zeigen eine Adenosin-5'-triphosphat (ATP) abhängige Aufnahme von Catecholaminen, welche Kriterien eines Energie-verbrauchenden Trägertransportes aufweist. Dieser Transport, zusammen mit einem intragranulären Speichermechanismus, erklärt wahrscheinlich die hohe Speicherkapazität der chromaffinen Granula für Catecholamine.
2. Andere Amine (z.B. 5-Hydroxytryptamin (5HT), Octopamin) werden ebenfalls durch die Membranen aufgenommen, aber in verschiedenem Masse. Dies erklärt wahrscheinlich, warum Amine, die normalerweise nicht in den Speicherorganellen vorkommen, in diesen accumulieren und als falsche Transmitter funktionieren können.
3. Auf dem Niveau der granulären Membran scheinen zwei Aufnahmemechanismen für Amine zu existieren, ein reserpinempfindlicher (durch den z.B. Catecholamine und 5HT transportiert werden) und ein reserpinresistenter (Transport von Tryptamin und z.T. von Metaraminol). Beide Mechanismen sind von ATP abhängig.
4. Chlorpromazin interferiert mit dem reserpinsensitiven und -resistenten Mechanismus der Noradrenalin (NA)-aufnahme. Deshalb scheinen die Wirkungsmechanismen von Neuroleptica und Reserpin in bezug auf Hemmung der Aminaufnahme durch Granulamembranen verschieden zu sein.
5. Es besteht nur unvollständige Parallelität zwischen Hemmung der NA-Aufnahme in Granulamembranen und Beschleunigung des DA-Turnovers in vivo durch Neuroleptica. Deshalb müssen noch andere Faktoren als Hemmung des Amintransportes in den Granulamembranen für die durch Neuroleptica bewirkte Beschleunigung des DA-Turnovers in vivo verantwortlich sein.

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