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DISCRIMINATION OF MONOAMINE UPTAKE BY MEMBRANES OF ADRENAL CHROMAFFIN GRANULES

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1 The accumulation of various radioactive monoamines by isolated membranes of bovine adrenal chromaffin granules was measured by equilibrium dialysis.

2 Adenosine-5′-triphosphate (ATP) in the presence of Mg^{++} stimulated the uptake of all the amines tested, but the accumulation of dopamine, (−)-noradrenaline (NA), 5-hydroxytryptamine (5-HT), (±)-adrenaline and (±)-octopamine was greater than that of tyramine, (±)-metaraminol, tryptamine, β-phenylethylamine and histamine.

3 At the higher concentration levels of the amines in the medium the ATP-dependent accumulation of dopamine, NA, adrenaline and 5-HT in the membranes reached a saturation level, whereas in the absence of the nucleotide no saturation level was attained.

4 Octopamine and 5-HT competitively inhibited the ATP-dependent uptake of NA.

5 Decrease in the incubation temperature or the presence of N-ethylmaleimide greatly reduced the ATP-stimulated amine accumulation. Ouabain had no effect on uptake.

6 Reserpine virtually abolished the ATP-dependent uptake of dopamine, NA and 5-HT, caused a partial inhibition of the metaraminol, octopamine and tyramine accumulation, but did not interfere with the uptake of tryptamine.

7 The content of endogenous catecholamines of the membranes was changed very little by incubation of NA and 5-HT in the presence of ATP. However, the membranes lost over 80% of their endogenous amines if incubated for 30 min without ATP.

8 The ATP content of the medium progressively decreased during the incubation of granular membranes.

9 It is concluded that the membrane of adrenal chromaffin granules discriminates between the various monoamines with regard to the magnitude of their uptake and that two mechanisms of ATP-stimulated uptake, one responsive and the other resistant to reserpine, exist at the level of this membrane. The ATP-stimulated transport at the granular membrane level may be an important factor in determining the intraneuronal storage of a physiological or false neurotransmitter.

Introduction

The amine storage organelles, e.g. of noradrenergic and 5-hydroxytryptaminergic neurones, are able to take up and accumulate amines other than those which they contain under physiological conditions. For instance, in the central nervous system L-DOPA causes an accumulation of dopamine not only in dopaminergic but also in 5-hydroxytryptaminergic neurones (Bartholini, Da Prada & Pletscher, 1968; Ng, Chase, Colburn & Kopin, 1970), and α-methyl-dopa leads to an increase of α-methyl-dopamine and α-methyl-noradrenaline in catecholaminergic neurones (Muscholl, 1972). Experiments with intact isolated amine storage granules, e.g. from blood platelets (Da Prada & Pletscher, 1968) or adrenal medulla (Carlsson, Hillarp & Waldeck, 1963) showed that the various monoamines are not taken up to the same extent. This may be due to differences in the aggregation of the amines with nucleotides (especially adenosine-5′-triphosphate, ATP) and bivalent cations present within the organelles. In fact, such differences have been shown to exist in artificial solutions (Berneis, Da Prada & Pletscher, 1969). However, it is not known whether in addition the granular membrane has the ability to discriminate between the various amines with regard to their uptake.

In the present work the uptake of different

monoamines by isolated membranes of bovine adrenal chromaffin granules is compared by a new method of equilibrium dialysis.

Methods

Preparation of membranes

Chromaffin granules were isolated from bovine adrenal glands (Smith & Winkler, 1967) with a post mortem delay of about 30 minutes. The isolated granules, obtained from 5-8 g medullary tissue, were lysed by hypotonic shock in 10 ml 0.015 M KCl and their membranes isolated by centrifugation at 39,000 x *g* for 10 minutes. After one washing of the sedimented membranes in 10 ml hypotonic KCl, they were resuspended in 7 ml 0.15 M KCl and dialysed for 20 min against 4 litres 0.15 M KCl using a b/HFD 1/20 Mini beaker dialyzer with cellulose hollow fibres (Bio-Rad Labs, Richmond, Cal., USA). Subsequently, the membranes were again sedimented by centrifugation and washed twice with 10 ml 0.15 M KCl. After suspending the membranes for the second washing the protein content was roughly estimated by measuring the light absorption of the suspension at 280 nm. A portion of the membranes was analysed for endogenous ATP and catecholamines. The remaining membranes were resuspended in 4-8 ml of 50 mM Na-glycerophosphate buffer, pH 7.4, containing 0.1 mM ascorbic acid, 5 mM $MgCl_2$ and 10 μM pargyline (*N*-benzyl-*N*-methyl-propargylamine, an inhibitor of monoamine oxidase; Pletscher, Gey & Burkard, 1966) (Taugner, 1971) so as to obtain a final concentration of about 800 μg protein per ml. In some experiments the buffer was supplemented with 5 mM ATP. All procedures were performed at 4°C.

Equilibrium dialysis

A 'Dianorm' equilibrium dialyzer (Diachema AG, Birmensdorf/Zürich, Switzerland), made of Teflon and containing two microchambers, separated by a 'spectrapor 2 T M membrane' (mol. wt cutoff: 12,000-14,000) (Spectrum Medical Industries, Inc., Los Angeles) (Figure 1) was used for all experiments. An aliquot of 125 μl of the final membrane suspensions was placed in one chamber and the same volume of buffer devoid of membranes in the other. Equal amounts of the radioactive amines, in a volume of 10 μl, were injected into each chamber.

Equilibrium dialysis experiments were carried out at various temperatures, incubation times and amine concentrations. At the end of an experiment, 40 μl aliquots, in duplicate, were extracted from each chamber, placed in 10 ml of scintillation mixture (Aquasol, New England Nuclear, Boston, USA) and counted in an Isokap/300 liquid scintillation system (Nuclear, Chicago, USA). The difference between the counts in the chamber containing the biological membranes and those in the chamber without membranes represents the amount of the amine incorporated into the membrane preparation. The quantity of the amine taken up by the membranes was expressed in nmol per mg protein. Virtually no radioactive amines were adsorbed onto the artificial membrane and the Teflon walls of the chambers.

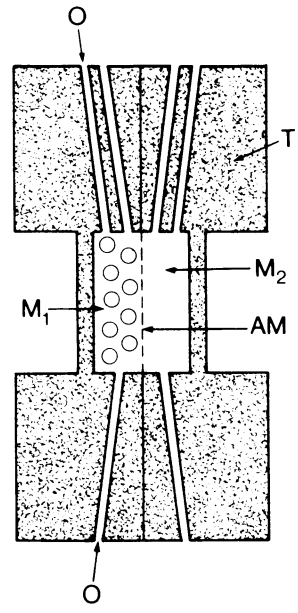

Figure 1 Model of microdialyzer. AM = semi-permeable artificial membrane; M_1 = microchamber (200 μl) containing biological membranes; M_2 = microchamber (200 μl) containing incubation medium only; O = open outlet (plugged during incubation) for filling or removal of incubation mixture; T = Teflon. After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

Assay methods

Endogenous catecholamines were determined by spectrophotofluorimetry (Laverty & Taylor, 1968). The ATP of the membranes (about 400 μg proteins) was extracted three times with 0.5 ml $HClO_4$, adjusted to pH 6.0 with K_2CO_3, injected

into a Varian Aerograph LCS 1000 ion exchange liquid chromatograph (Varian, California, USA) (Brown, 1973) and measured by absorption at 254 nm. In some experiments this method was also used for determining the content of ATP, adenosine-5′-diphosphate (ADP) and adenosine-5′-monophosphate (AMP) of the medium which was injected into the aerograph after dilution with 0.1 M phosphate buffer, pH 6.0. In other experiments the determination of ATP in the medium was carried out by the luciferin-luciferase method (Holmsen, Holmsen & Bernhardsen, 1966). Proteins were estimated colorimetrically (Lowry, Rosebrough, Farr & Randall, 1951).

Materials

Adenosine-5′-triphosphate disodium salt, Sigma grade, was obtained from Sigma, St Louis, Mo., USA; (±)-adrenaline-[carbinol-^{14}C] (±)-bitartrate (50 mCi/mmol), 3,4-dihydroxyphenyl[ethylamine-2-^{14}C] hydrochloride (55 mCi/mmol), 5-hydroxytryptamine-[3′-^{14}C]-creatinine sulphate (55 mCi/mmol), histamine-[ring-2-^{14}C] dihydrochloride (54 mCi/mmol), (−)-noradrenaline-[carbinol-^{14}C] (±)-bitartrate (37 mCi/mmol) and tyramine-[1-^{14}C] hydrochloride (43.7 mCi/mmol) from the Radiochemical Centre, Amersham, Bucks.; (±)-metaraminol-[7-^{3}H] (N) (5 Ci/mmol), (±)-octopamine-[2-^{3}H] (N) (1 Ci/mmol), tryptamine-[2-^{14}C]-bisuccinate (10 mCi/mmol) and β-phenylethylamine-[1-^{14}C] hydrochloride (7 mCi/mmol) from New England Nuclear, Boston, USA. All other substances used were of analytical grade. The purity of the radioactive compounds indicated by the suppliers was confirmed by paper chromatography.

Results

Noradrenaline

The accumulation of (−)-noradrenaline (NA) in the isolated membranes of chromaffin granules was greatly stimulated by the presence of ATP in the incubation medium. The uptake increased with the time of incubation and reached a maximum between 30 and 60 minutes. Thereafter, the amounts of labelled NA in the membranes decreased, but were still elevated at 150 min (Figure 2). In the absence of ATP, the amount of NA which accumulated was greatly reduced (Table 1), and no maximum occurred at 30-60 minutes.

The uptake of NA during the first 30 min also rose with increasing concentration of the radioactive amine. In the presence of ATP, a saturation level was reached at amine concentrations of 200-400 μM. Without ATP, the NA showed much less accumulation in the membrane over the whole concentration range (10-600 μM). The saturation level of NA in the presence of ATP was about 100 times higher than the corresponding amine levels obtained in the absence of the nucleotide (Figure 3).

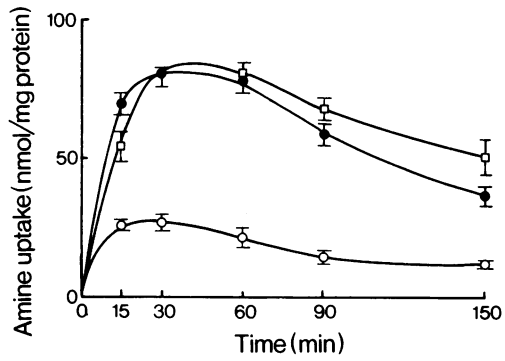

Figure 2 Time course of the uptake of labelled (−)-noradrenaline (□), dopamine (●) and tryptamine (○) by isolated membranes of bovine adrenal chromaffin granules incubated at 37° C in $MgCl_2$-containing medium in the presence of adenosine-5′-triphosphate (ATP). Initial concentrations: amines 45 μM, ATP 5 mM. The points are mean of 3-8 experiments. Vertical bars show s.e. mean.

Other amines

The accumulation by membranes of adrenal chromaffin granules of all the other amines tested was stimulated by ATP. The magnitude of the ATP-stimulated uptake, however, showed considerable differences (Table 1). For instance, dopamine, NA, 5-HT, (±)-adrenaline and (±)-octopamine were taken up to a greater extent than tyramine, tryptamine, (±)-metaraminol, phenylethylamine and histamine. The accumulation of dopamine and histamine differed by a factor of about 15.

The uptake of dopamine and tryptamine in the presence of ATP followed a time course similar to that of NA, i.e. it reached a maximum after 30-60 min with a subsequent drop (Figure 2). Other amines, e.g. 5-HT, behaved similarly. The small uptake which occurred in the absence of ATP did not exhibit this maximum. Dopamine, 5-HT and adrenaline also showed a concentration-dependence similar to NA. Thus, in the presence of ATP a saturation level was attained at 200-400 μM, whereas in the absence of ATP a

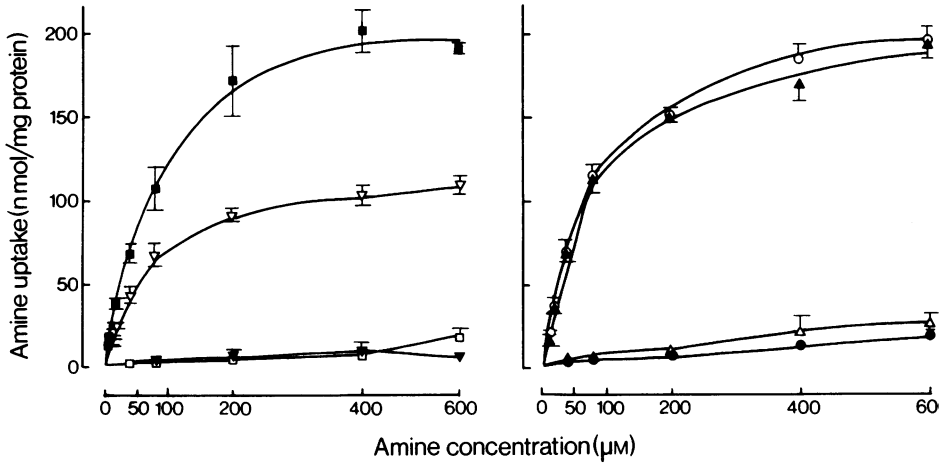

Figure 3 Uptake of labelled (−)-noradrenaline (NA), (±)-adrenaline (Ad), dopamine (DA) and 5-hydroxytryptamine (5-HT) by isolated membranes of bovine adrenal chromaffin granules incubated at 37° C for 30 min in $MgCl_2$-containing medium with various concentrations of the amines in the presence and absence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

much less marked uptake occurred over the whole concentration range (Figure 3).

Both octopamine (Figure 4) and 5-HT inhibited the uptake of NA by adrenal granular membranes. According to the double reciprocal plots, i.e. $1/V$ versus $1/(S)$ (Figure 4) and V versus $V/(S)$, the inhibition was of the competitive type. The K_m for NA amounted to 11×10^{-5}, the K_i for octopamine and 5-HT to 17×10^{-5} and 3.5×10^{-5}, respectively.

Interference with ATP-stimulated uptake

The ATP-stimulated uptake of all the monoamines tested decreased considerably when the temperature of the incubation medium was diminished,

Table 1 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 with	*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
Tryptamine	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'-triphosphate (ATP) 5 mM. The figures represent mean with s.e. mean of 3-6 experiments and are indicated in nmol per mg membrane protein.

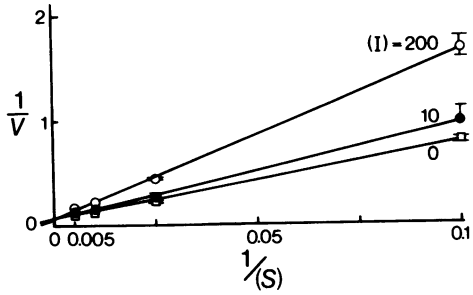

Figure 4 Double reciprocal plot (Lineweaver-Burk) of the effect of octopamine on the uptake of (−)-[^{14}C]-noradrenaline (NA) by membranes of adrenal chromaffin granules incubated for 10 min at 37° C in $MgCl_2$-containing medium in the presence of ATP (initial concentration 5 mM). *V* = initial velocity of uptake (nmol per mg of protein per 10 min); *S* = molar concentration of NA. (I) = concentration of octopamine in μM. Each value is the mean of 4-8 experiments. Vertical bars show s.e. mean.

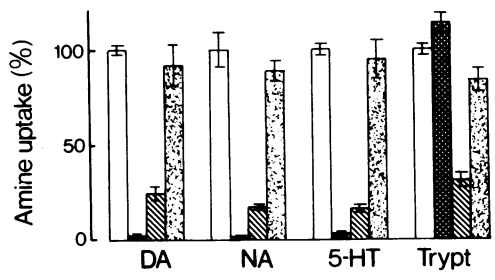

Figure 5 Effect of reserpine (10^{-5} M; cross-hatched), N-ethylmaleimide (diagonally hatched; 5 x 10^{-4} M) and ouabain (5 x 10^{-4} M; stippled) on the uptake of labelled dopamine (DA), (−)-noradrenaline (NA), 5-hydroxytryptamine (5-HT) and tryptamine (Trypt) by isolated membranes of bovine adrenal chromaffin granules incubated for 30 min at 37° C in $MgCl_2$-containing medium in the presence of ATP (initial concentration 5 mM). Open columns, controls. The columns are mean of 3-5 experiments expressed as percentage of the values obtained in controls, not incubated with inhibitors (=100%). Vertical bars show s.e. mean.

the accumulation in the membranes at 2° C approximately corresponding to that seen at 37° C in the absence of ATP (Table 2).

N-ethylmaleimide, but not ouabain, greatly inhibited the ATP-dependent uptake of dopamine, 5-HT, NA and tryptamine (Figure 5).

The action of reserpine on the ATP-stimulated uptake differed for the various amines. The drug caused a dose-dependent inhibition of the accumulation of NA, octopamine and metaraminol. However, the inhibition of the uptake of NA was considerably greater than that of metaraminol and slightly more marked than that of octopamine (Figure 6). Dopamine and 5-HT behaved like NA (Figure 5) and tyramine like metaraminol. In contrast, reserpine in concentrations as high as 10^{-5} M did not decrease the uptake of tryptamine (Figure 5).

Endogenous catecholamines and ATP

In three experiments, the content of endogenous catecholamines in the washed membranes

Table 2 Temperature dependence of uptake of labelled amines by isolated membranes of bovine adrenal chromaffin granules incubated in a $MgCl_2$-containing medium for 30 minutes

	Incubation temperature, °C		
Incubation with	*2*	*25*	*37*
Dopamine	2.7 ± 1.3	50.9 ± 8.4	80.09 ± 7.26
(−)-Noradrenaline	0.8 ± 0.2	34.1 ± 6.4	76.73 ± 5.56
5-Hydroxytryptamine	3.4 ± 0.9	56.1 ± 8.6	74.04 ± 7.20
(±)-Adrenaline	0.8 ± 0.2	25.0 ± 7.7	64.64 ± 6.77
(±)-Octopamine	1.2 ± 0.2	19.6 ± 4.4	54.02 ± 3.39
Tyramine	1.2 ± 0.2	34.6 ± 3.1	37.93 ± 3.08
(±)-Metaraminol	0.3 ± 0.3	9.2 ± 2.9	28.86 ± 0.93
Tryptamine	4.0 ± 0.0	19.2 ± 0.3	22.47 ± 0.77

Initial concentrations in incubation medium: amines 45 μM, adenosine-5'-triphosphate 5 mM. The values represent mean with s.e. mean of 3 experiments and are indicated in nmol per mg membrane protein.

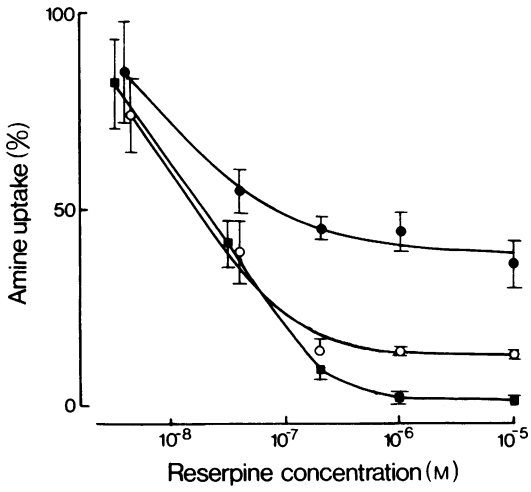

Figure 6 Effect of various concentrations of reserpine on the uptake of labelled (−)-noradrenaline (■), (±)-octopamine (○) and (±)-metaraminol (●) at 37° C by isolated chromaffin granular membranes incubated for 30 min at 37° C in $MgCl_2$-containing medium in the presence of ATP. Initial concentrations: labelled amines 45 μM, ATP 5 mM. The points are mean 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%). The uptake of NA, octopamine and metaraminol in the absence of ATP amounted to 4 ± 1%, 2 ± 2% and 1 ± 1% respectively compared to the controls. Vertical bars show s.e. mean.

amounted to 86.6 ± 8.5 nmol/mg protein. The amine level was not significantly changed ($P > 0.01$) by incubation of the membranes at 37° C for 30 min in the presence of ATP, but decreased to 17.2 ± 1.0% (three experiments) during incubation for 30 min in the absence of ATP. On incubation of membranes for 30 min at 37° C with 45 μM NA or 5-HT in the presence of

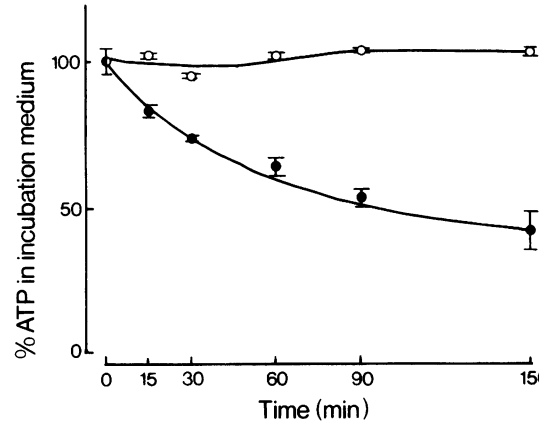

Figure 7 Decrease of the ATP content of the medium during incubation at 37° C with adrenal chromaffin membranes. (○) Without membranes; (●) with membranes. Initially the medium contained ATP 5 mM and $MgCl_2$ 5 mM. The points are mean of 5-6 experiments and are indicated as percentages of the 0 values (=100%). Vertical bars show s.e. mean. The measurements of ATP were carried out with the luciferin-luciferase method (see methods section).

ATP their endogenous catecholamine content changed very little (Table 3).

The concentration of endogenous ATP in the washed membranes amounted to 4.56 ± 0.27 nmol per mg protein (three experiments).

Degradation of ATP

In suspensions of isolated membranes incubated in the presence of ATP 5 mM the content of this nucleotide decreased with time (Figure 7). After 150 min the incubation medium contained only 2.11 ± 0.32 μmol ATP/ml (initial concentration 5 mM), but 2.23 ± 0.03 μM ADP and 0.36 ± 0.02 mM AMP were also present (three

Table 3 Effect of (−)-[^{14}C]-noradrenaline (NA) and [^{14}C]-5-hydroxytryptamine (5-HT) (initial concentration both 45 μM) on endogenous catecholamines (CA) of chromaffin granular membranes incubated at 37°C for 30 min in a $MgCl_2$-containing medium in the presence of adenosine-5'-triphosphate (5 mM)

	Amines in membrane (nmol/mg protein)			
Incubation with	*0 min*		*30 min*	
	Endogenous CA	*Total CA*	*^{14}C-amines*	*Endogenous CA*
[^{14}C]-NA	105.9 ± 5.4	239.0 ± 14.0	120.1 ± 5.0	118.9 ± 26.0
[^{14}C]-5-HT	105.9 ± 5.4		110.1 ± 6.6	86.0 ± 4.5

In the experiments with NA the amount of endogenous CA after 30 min was calculated by subtracting the labelled amine from the total CA. Each figure indicates mean with s.e. mean of 4 experiments.

experiments). Addition of dopamine 45 μM to the ATP-containing incubation medium had little effect on the decrease of the ATP content.

Discussion

The method of equilibrium dialysis used in the present work for measuring the uptake of biogenic amines by membranes of adrenal chromaffin granules proved to be simpler and more sensitive than previously applied techniques. Thus, the amounts of membranes used (about 100 μg protein per experiment) were smaller than in earlier experiments, and no separation and washing of the membranes had to be carried out at the end of the incubation period. Furthermore, in the presence of ATP the uptake of NA (initial concentration 45 μM) was considerably higher than that seen previously (about 80 versus 10 nmol per mg membrane protein) (Table 1) (Taugner, 1972).

The diminution of the amine uptake which followed the maximum amine accumulation after 30-60 min (Figure 2) might be connected with damage to the membranes caused by prolonged incubation in an artificial medium. This is confirmed by preliminary experiments where it was found that after preincubation at 37°C for 180 min in the presence of ATP 5 mM, the membranes reincubated in fresh medium showed a decrease of their dopamine uptake to 17 ± 4% (n = 3) compared to non-preincubated controls (=100%). The amine uptake may also be diminished by decrease of the ATP concentration in the medium in the course of the incubation (Figure 7) and by accumulation of ADP which has been shown to inhibit the ATP-stimulated NA uptake (unpublished results). The measurements of the ATP-dependent amine uptake must therefore be carried out during the initial period of incubation (e.g. after 15-30 minutes).

The uptake results with radioactive NA and adrenaline described above agree with previous findings which strongly suggested the existence in granular membranes of a specific ATP- (and Mg^{++}-) sensitive mechanism besides a less specific and much less efficient process independent of ATP (Taugner, 1972). The present experiments indicate that the content of endogenous catecholamines in adrenal granular membranes also depended on ATP. In fact, incubation of the membranes at 37°C for 30 min in media devoid of ATP decreased the membrane content of endogenous amines to one-sixth (compared to preincubation values), whereas in the presence of ATP little diminution was to be seen.

Several other amines (Table 1) behaved like NA and adrenaline with regard to their accumulation by membranes of chromaffin granules. In fact, in Mg^{++}-containing medium the uptake of these amines (like that of NA and adrenaline) was stimulated by ATP, followed saturation kinetics, was inhibited by a decrease in temperature, by N-ethylmaleimide and in part also by reserpine, whereas ouabain had little effect. Moreover, amines such as octopamine and 5-HT competitively inhibited the ATP-stimulated uptake of NA. These findings indicate that membranes of chromaffin granules also accumulate 'foreign' amines by a specific transport process and that the energy used for this uptake probably derived from the hydrolysis of ATP by the Mg^{++}-dependent ATPase present in the membrane (Hillarp, 1958). The exact mechanism of this transport is not known. It has been suggested that the ATP-ATPase system acts by transphosphorylation of a hypothetical membrane carrier (Slotkin, 1973).

The ATP-stimulated uptake of NA and 5-HT by the granular membranes occurred without a marked concomitant decrease of the endogenous catecholamines. Therefore, the accumulation of the major part of the radioactive amines was not due to an exchange with the endogenous catecholamines, but to a net amine uptake. The site of accumulation of the amines in the membrane preparations in the presence of ATP cannot be established from the results of the present experiments. However, according to previous findings the majority of NA seemed to accumulate in the interior of the membrane vesicles (newly formed from membrane fragments), whereas only minor amounts were bound to the membranes (Agostini & Taugner, 1973).

These findings together with previous results (Da Prada, Berneis & Pletscher, 1971) indicate that ATP has a double function in the accumulation of amines in storage organelles. The nucleotide located within the organelles forms a storage complex by aggregation with the amines, bivalent cations and possibly proteins (chromogranins) (Berneis, Goetz, Da Prada & Pletscher, 1973), whereas another part of ATP is involved in a specific transport of the amines at the level of the granular membranes. It is of interest that there were considerable differences in the ATP-stimulated uptake of the various amines by the granular membranes. Amines like dopamine, NA, 5-HT, whose physiological concentration in certain neurones (e.g. of mammalian brain) is very high, accumulated to a greater extent than others such as histamine, phenylethylamine, tryptamine and tyramine. Therefore, the transport at the granular membrane level might be an important factor in determining the degree of accumulation of a physiologically occurring amine or of a false

transmitter in storage organelles of the peripheral sympathetic nervous system and the brain. In this connection it may be of interest that octopamine, whose uptake was stimulated by ATP in the present experiments, accumulated *in vivo* after inhibition of monoamine oxidase and has been claimed to function as a false neurotransmitter (Kopin, Fisher, Musacchio, Horst & Weise, 1965).

The reason why, in the presence of ATP, the granular membrane takes up various amines to a different degree remains to be investigated. The existence of differences in the stereochemical conformation of the amines with regard to that of a hypothetical carrier or receptor is a possibility to be considered.

The above data on the uptake of the amines may in part need some correction. Thus, according to preliminary results some dopamine was transformed into NA (about 50%) during the incubation, and a partial transformation of tyramine to octopamine by adrenal granular membranes (which contain dopamine-β-hydroxylase) has also been shown. However, the presence of a β-hydroxy group was probably not a major requirement for uptake since both NA and dopamine accumulated to about the same extent although the latter was only partially β-hydroxylated. Another source of error may result from the comparison of the (−)-form of NA with the racemates of adrenaline, metaraminol and octopamine. Thus, earlier experiments with (−) and (+)-NA (Taugner, 1972) indicated that the uptake of the enantiomers by chromaffin granular membranes may be somewhat different in magnitude.

In previous experiments, reserpine administered *in vivo* has been shown to interfere partially with the granular uptake of metaraminol (Almgren & Lundborg, 1970). The present results clearly demonstrate that reserpine also partially inhibits the metaraminol uptake *in vitro* and that the reserpine-sensitive mechanism is located in the granular membrane. On the other hand, reserpine interfered less with the uptake of metaraminol than with that of NA, dopamine and 5-HT. This finding confirms earlier results according to which the granular uptake of metaraminol showed partial reserpine resistance (Lundborg, 1966; Lundborg & Stitzel, 1967; Almgren & Lundborg, 1970; Slotkin & Kirshner, 1971). In addition, the present experiments showed that tyramine and to a small extent octopamine seemed to be partly reserpine-resistant, whereas tryptamine was totally resistant to the alkaloid.

The nature of the reserpine-resistant uptake is not known. However, the present experiments indicate that this uptake is stimulated by ATP. In fact, in media containing relatively high concentrations of reserpine (10^{-5} M) the accumulation of metaraminol, octopamine and tryptamine in granular membranes was enhanced by the nucleotide (compare Figures 5 and 6 with Table 1). The mechanism of action of reserpine in inhibiting the amine uptake is also uncertain. In previous experiments, evidence has been presented that reserpine might interfere with a specific transport ATPase located in the adrenal granular membranes (Taugner & Hasselbach, 1966). If this were the case, one would have to postulate that two or more ATPases with different reserpine sensitivity are involved in the transport of the various amines.

In conclusion, membranes of bovine adrenal chromaffin granules take up amines other than those contained physiologically in these organelles by a specific transport mechanism. However, the magnitude of this uptake and the degree of its inhibition by reserpine differ for the various amines. The transport at the level of the granular membranes together with other mechanisms (e.g. intragranular aggregation, transport through the neuronal membrane) may be an important factor in determining the intraneuronal accumulation of physiological and false neurotransmitters.

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