Biochem. J. (1983) **210**, 789–794
Printed in Great Britain

Specificity and properties of the nucleotide carrier in chromaffin granules from bovine adrenal medulla

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(*Received 5 April 1982/Accepted 30 November 1982*)

1. The influence of various substances on the uptake of [^{3}H]ATP and [^{14}C]-noradrenaline into isolated bovine chromaffin granules was investigated. The carrier-mediated [^{3}H]ATP uptake is specifically inhibited by SO_4^{2-}, PO_4^{3-} and phosphoenolpyruvate. Compounds with carboxylic acid or sulphonic acid groups had no significant inhibitory effects on either uptake. 2. $^{35}SO_4^{2-}$, $^{32}PO_4^{3-}$ and phosphoenol[^{14}C]pyruvate are taken up into chromaffin granules by a temperature-dependent process that is inhibited by atractyloside, uncouplers of oxidative phosphorylation and lipid-permeant anions. The apparent K_m of $^{35}SO_4^{2-}$ uptake is 0.4mM. 3. These results indicate that the nucleotide carrier in chromaffin granules has a broad specificity, transporting compounds with two strong negative charges. 4. Amino acid probes influence the uptake of ATP and catecholamines differently. Pyridoxal phosphate inhibits both uptake processes, 4,4′-di-isothiocyanostilbene-2,2′-disulphonic acid preferentially blocks ATP uptake, whereas phenylglyoxal blocks only ATP transport. It is suggested that the nucleotide carrier possesses arginine residues in a functionally important position. 5. The significance of these results obtained on isolated granules for the function of chromaffin granules within the cell is discussed.

Several storage organelles for hormones and neurotransmitters contain a high concentration of nucleotides (see Lagercrantz, 1976; Winkler & Westhead, 1980; Morris, 1980). Thus, in chromaffin granules, which store the catecholamines in the adrenal medulla, the intravesicular ATP concentration amounts to 0.15M. Radioactively labelled precursors can be incorporated into this ATP pool (Stjärne *et al.*, 1970; Stevens *et al.*, 1972; Winkler *et al.*, 1972) and evidence has been presented that this ATP is synthesized outside the chromaffin granules (Peer *et al.*, 1976). In accordance with this, isolated chromaffin granules possess an uptake system for ATP (Kostron *et al.*, 1977; Phillips & Morton, 1978; Aberer *et al.*, 1978). This uptake is saturable and inhibited by atractyloside. It is driven by the electrical part (Aberer *et al.*, 1978; Weber & Winkler, 1981) of an electrochemical proton gradient, which is provided in these organelles by a proton-pumping ATPase (see Njus & Radda, 1978). The carrier not only transports ATP, but also GTP and UTP (Weber & Winkler, 1981).

H. Grueninger & D. K. Apps (personal communication) have found that phosphoenolpyruvate is an inhibitor of nucleotide uptake into granule ghosts and this finding initiated the present study. It is demonstrated that the nucleotide carrier of chromaffin granules has a very broad specificity, transporting, in addition to nucleotides, phosphoenolpyruvate, phosphate and sulphate. The molecular properties of the carrier were tested with several probes for certain amino acids. A preliminary account of some of the findings has already been given (Grueninger *et al.*, 1980).

Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DIDS, 4,4′-di-isothiocyanostilbene-2,2′-disulphonic acid.

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Experimental

Materials

[2-^{3}H]ATP (ammonium salt; sp. radioactivity 15–30Ci/mmol), DL-[*methylene*-^{14}C]noradrenaline DL-bistartrate (sp. radioactivity 50Ci/mol); $Na_2{}^{35}SO_4$ (sp. radioactivity 30Ci/mol); [^{32}P]P_i

0306-3283/83/030789-06$2.00

(carrier-free in dilute HCl solution, pH 2–3; 6–50 mCi at 10 mCi/ml) and phosphoenol[1-^{14}C]pyruvate (cyclohexylammonium salt; sp. radioactivity 13 Ci/mol) were purchased from Amersham International (Amersham, Bucks., U.K.). [^{14}C]Noradrenaline was dissolved in 1 mM-HCl containing unlabelled 2 mM-noradrenaline bistartrate. DIDS was obtained from Calbiochem, La Jolla, CA, U.S.A. and Cibacron Blue (Reactive Blue 2), pyridoxal 5-phosphate and palmitoyl-CoA were from Sigma Chemical Co. All other chemical compounds were obtained from Sigma Chemical Co. and were of analytical grade. Thin-layer plates (Polygram Cell 300 PEI) were from Macherey-Nagel, Düren, Germany.

Methods

Isolation and incubation of chromaffin granules. Partly purified chromaffin granules from bovine adrenal were isolated by differential centrifugation as described previously (Kostron *et al.*, 1977; Aberer *et al.*, 1978). The resuspended chromaffin granules (2–4 mg of protein) were incubated in 1 ml of buffered (0.01 M-Tris/HCl, pH 7.2) 0.3 or 0.6 M-sucrose solution for 5 min at 37°C together with radioactively labelled compounds (10–20 μCi of [^{3}H]ATP, 5–10 μCi of phosphoenol[^{14}C]pyruvate, 10 μCi of $^{35}SO_4^{2-}$, 0.25 μCi of [^{14}C]noradrenaline or 100 μCi of $^{32}PO_4^{3-}$). After incubation the suspensions were diluted with 3 ml of cold (0°C) buffered 0.3 or 0.6 M-sucrose containing 20 mM-disodium EDTA. After sedimentation of the granules and one additional wash (see Weber & Winkler, 1981) purified granules were obtained by centrifugation through 1.5 M-sucrose solution (see Smith & Winkler, 1967) for 60 min and 120000 $g_{max.}$. The pellets of pure chromaffin granules were further analysed. Uptake rates were calculated as described by Weber & Winkler (1981). The supernatants of the first sedimentation step after incubation were always analysed for catecholamines. This enabled us to detect any lysis of chromaffin granules caused by the addition of the various compounds to the incubation medium.

Chemical assays. Protein was determined by the biuret method as described by Winkler *et al.* (1970). Catecholamines were measured colorimetrically (see Euler & Hamberg, 1949). In the experiments with phosphoenol[^{14}C]pyruvate the supernatants after incubations were analysed for the presence of [^{14}C]pyruvate with a column (5 ml volume) filled with Dowex, 2 resin (X8; 50–100 mesh). Elution was performed by increasing the concentration of Cl^- from 1 mM to 100 mM by using a linear gradient. Fractions (4 ml) were collected and portions were assayed for radioactivity and pH. [^{14}C]Pyruvate was well separated from phosphoenol[^{14}C]pyruvate by this method.

Results

Uptake of [^{3}H]ATP and [^{14}C]noradrenaline

The effects of various compounds on the uptake of [^{3}H]ATP and [^{14}C]noradrenaline into chromaffin granules are given in Table 1. Phosphoenolpyruvate, PO_4^{3-} and SO_4^{2-} produced a specific inhibition of nucleotide uptake. The highest concentration of phosphoenolpyruvate (10 mM) inhibited nucleotide uptake by 76% without any effect on noradrenaline uptake. Mono- and di-carboxylic acids slightly activated both uptakes. Compounds with sulphonic acid groups, i.e. isethionate, Hepes and taurate, either slightly activated or had no effect. Adenosine and adenine were without significant effects.

The influence of Cibacron Blue and palmitoyl-CoA on these transport processes was more complex. In lower concentrations Cibacron Blue inhibited mainly ATP uptake; however, at higher concentrations both uptake processes were influenced (see Table 2). Palmitoyl-CoA also led to a stronger inhibition of ATP uptake. Higher concentrations of this compound induced a significant lysis of the granules.

Table 1. *Uptake of [^{3}H]ATP and [^{14}C]noradrenaline into chromaffin granules*

Chromaffin granules were incubated with [^{3}H]ATP (2 mM) and [^{14}C]noradrenaline for 5 min at 37°C. The uptake rates in control tubes obtained in each experiment were taken as 100%. Maximum values were: 0.50 ± 0.02 nmol of [^{3}H]ATP/mg of protein per min ($n = 18$) and 3.6 ± 0.2 nmol of [^{14}C]noradrenaline/mg of protein per min ($n = 19$). The values are means ± S.E.M. The number of experimental values is given in parentheses.

	Uptake (%) of:	
	[^{3}H]ATP	[^{14}C]Noradrenaline
Control	100	100
Phosphoenolpyruvate		
(1 mM)	57 ± 2	109 ± 4 (4)
(5 mM)	33 ± 2	122 ± 3 (4)
(10 mM)	24 ± 1	109 ± 6 (6)
PO_4^{3-} (10 mM)	59 ± 3	127 ± 3 (5)
SO_4^{2-} (5 mM)	54 ± 1	92 ± 3 (8)
Pyruvate (10 mM)	121 ± 3	119 ± 7 (6)
L-Glutamate (10 mM)	120 ± 3	120 ± 7 (6)
Acetate (10 mM)	126 ± 4	119 ± 5 (6)
Isethionate (10 mM)	102 ± 3	121 ± 2 (6)
Taurate (10 mM)	100 ± 3	111 ± 2 (4)
Hepes (10 mM)	101 ± 2	128 ± 3 (5)
Adenine (5 mM)	107 ± 4	101 ± 8 (4)
Adenosine (2 mM)	101 ± 4	92 ± 5 (5)

Table 2. *Effects of Cibracon Blue and palmitoyl-CoA on uptake into chromaffin granules*

Chromaffin granules were incubated as described in the legend to Table 1. The results are expressed as percentages of the uptake rates in control tubes, which were taken as 100%. Values are means ± S.E.M. The number of experimental values is given in parentheses.

	Uptake (%) of:	
	[^{3}H]ATP	[^{14}C]Noradrenaline
Control	100	100
Cibacron Blue		
(5 μM)	70	94 (2)
(10 μM)	56 ± 8	92 ± 3 (7)
(50 μM)	25 ± 3	64 ± 3 (6)
Palmitoyl-CoA		
(5 μM)	88 ± 2	94 ± 2 (6)
(10 μM)	52 ± 1	77 ± 1 (4)

Uptake of phosphoenol[^{14}C]pyruvate and $^{32}PO_4^{3-}$ into chromaffin granules

Both compounds are taken up into the granules by a temperature-dependent process (see Table 3). Blockers of ATP uptake, i.e. atractyloside, an uncoupler of oxidative phosphorylation (CCCP) and SCN^- (a lipid-permeant anion) inhibited this uptake. To ensure that during incubation phosphoenolpyruvate was not broken down into [^{14}C]-pyruvate and that this compound was subsequently taken up into the granules, the medium after incubation was analysed by ion-exchange chromatography. No significant amounts of [^{14}C]pyruvate could be detected.

Kinetic analysis of $^{35}SO_4^{2-}$ uptake into chromaffin granules

Table 4 presents the effect of various compounds on the uptake of $^{35}SO_4^{2-}$ into chromaffin granules. Compounds that are also blockers of [^{3}H]ATP uptake led to a nearly complete inhibition of $^{35}SO_4^{2-}$ uptake. In these experiments incubation time was 5 min. However, the rate of $^{35}SO_4^{2-}$ uptake was linear up to 15 min (results not shown).

An analysis of the uptake rates in relation to the $^{35}SO_4^{2-}$ concentration in the medium is given in Fig. 1. The uptake rates levelled off at substrate concentrations above 2 mM (curve A, Fig. 1). In the presence of SCN^- the electrical part of the proton gradient and therefore the driving force for the carrier-mediated uptake is abolished (see Weber & Winkler, 1981). Under these conditions $^{35}SO_4^{2-}$ entered chromaffin granules by a diffusion process that increased in rate linearly with rising substrate concentrations (see curve B, Fig. 1). When this diffusion component is subtracted from the total uptake, the carrier-mediated uptake is obtained (curve A−B, Fig. 1). A Lineweaver–Burk plot of these uptake rates gives an apparent K_m of 0.4 mM and a $V_{max.}$ of 0.5 nmol/mg of protein per min.

In these experiments no ATP was added to the incubation medium. To test for the effect of exogenous ATP [cf. Weber & Winkler (1981) for catecholamine uptake] chromaffin granules were incubated (5 min) with 10 mM-$^{35}SO_4^{2-}$ in the presence or absence of ATP (2 mM) and Mg^{2+} (1 mM). The high concentration of $^{35}SO_4^{2-}$ was used to

Table 3. *Uptake of [^{14}C]phosphoenolpyruvate and $^{32}PO_4^{3-}$ into chromaffin granules*

Chromaffin granules were incubated with 1 mM-phosphoenol[^{14}C]pyruvate (PEP) or 1 mM-$^{32}PO_4^{3-}$ for 5 min at 37°C. The uptake rates in control incubations were taken as 100%. The maximum rates in control incubations were taken as 100%. The maximum rate for phosphoenol[^{14}C]pyruvate (1 mM) was 0.66 nmol/mg of protein per min (±0.04; $n = 11$). Maximum PO_4^{3-} uptake rates were not calculated because ATP release from the granules during incubation and PO_4^{3-} formation from ATP breakdown made determination of the PO_4^{3-} concentration in the medium unreliable. The experiments with SCN^- were performed in the presence of 0.6 M-sucrose (see the legend to Fig. 1). Values are means ± S.E.M. The number of experimental values is given in parentheses.

	Uptake (%) of:	
	Phosphoenol-[^{14}C]pyruvate	$^{32}PO_4^{3-}$
Control	100	100
Atractyloside (0.2 mM)	33 ± 2 (4)	40 ± 2 (4)
CCCP (125 μM)	55 ± 1 (5)	—
SCN^- (40 mM)	10 ± 1 (5)	26 (2)
0°C	5 (2)	11 ± 1 (4)

Table 4. *$^{35}SO_4^{2-}$ uptake into chromaffin granules*

Chromaffin granules were incubated with $^{35}SO_4^{2-}$ (1 mM) for 5 min at 37°C. The uptake rates in the presence of various compounds are given as percentages of controls, which were taken as 100%. The maximum uptake rate of the control was 0.38 nmol of $^{35}SO_4^{2-}$/mg of protein per min (±0.02; $n = 8$). Values are means ± S.E.M. The number of experimental values is given in parentheses. The experiments with SCN^- were performed in the presence of 0.6 M-sucrose (see the legend to Fig. 1).

	Uptake of $^{35}SO_4^{2-}$ (%)
Control	100
Atractyloside (0.2 mM)	15 ± 0 (3)
Phosphoenolpyruvate (10 mM)	8 ± 0.3 (6)
SCN^- (40 mM)	9 ± 1 (3)
At 0°C	2 ± 0.2 (5)

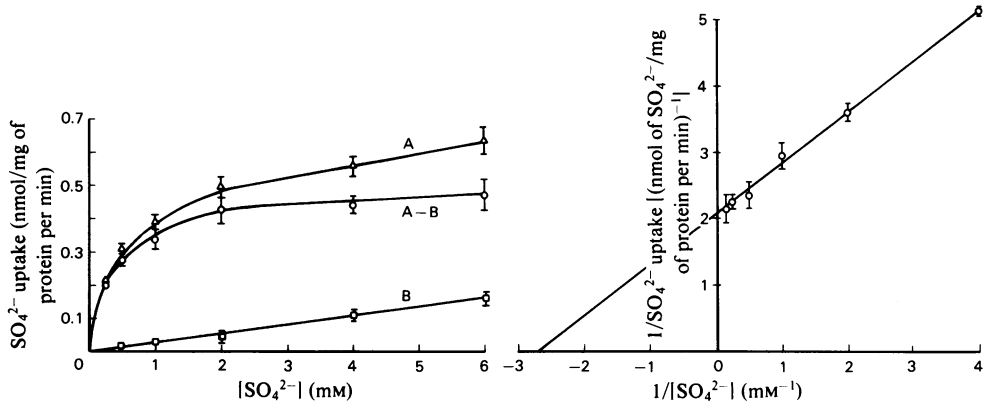

Fig. 1. *Kinetic analysis of $^{35}SO_4^{2-}$ uptake into chromaffin granules*

Chromaffin granules were incubated with $^{35}SO_4^{2-}$ (0.5–6 mM) for 5 min at 37°C with and without SCN^- (40 mM). To avoid lysis of the granules owing to the entry of SCN^- into them (see Casey *et al.*, 1976), 0.6 M-sucrose was used in these experiments. Uptake rates for the carrier-mediated transport were obtained by subtracting the uptake rates in the presence of SCN^- (B) from those in its absence (A). A Lineweaver–Burk plot of the carrier-mediated transport rates is shown on the right. The results are means ± S.E.M. ($n = 3$).

Table 5. *Influence of amino acid probes on uptake into chromaffin granules*

Chromaffin granules were incubated with 2 mM-[^{3}H]ATP and [^{14}C]noradrenaline for 5 min at 37°C. The results are expressed as percentages of control uptake rates, which were taken as 100%. For phenylglyoxal (5 µl of an ethanolic solution added to incubation medium) the granules were pre-incubated with this compound for 15 min at 4°C. In these experiments a slight increase of catecholamine leakage of the granules occurred, amounting to about 10% of the total catecholamine content. Values are means ± S.E.M. The number of experiments is given in parentheses.

	Uptake (%) of:	
	[^{3}H]ATP	[^{14}C]Noradrenaline
Control	100	100
Phenylglyoxal		
(5 mM)	60 ± 1	90 ± 3 (7)
(10 mM)	45 ± 2	91 ± 2 (9)
(20 mM)	36 ± 2	108 ± 5 (5)
DIDS		
(1 µM)	48 ± 1	102 ± 2 (3)
(5 µM)	26 ± 1	91 ± 3 (5)
(10 µM)	23 ± 1	84 ± 1 (6)
Pyridoxal phosphate (1 mM)	55 ± 2	45 ± 1 (4)
7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (250 µM)	20 ± 2 (5)	32 ± 2 (5)
N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (1 mM)	55 ± 4 (6)	70 ± 10 (4)
2-Hydroxy-5-nitrobenzyl bromide (100 µM)	76 ± 1 (6)	39 ± 1 (6)

reduce interference from ATP by competition for the carrier (see the Discussion section). The total uptake rates were corrected for the diffusion component (in the presence of 40 mM-SCN^- as shown above). The corrected uptake rate in the absence of ATP was 0.45 ± 0.02 nmol of $^{35}SO_4^{2-}$/mg of protein per min ($n = 6$; mean ± S.E.M.), whereas in the presence of ATP it was 0.62 ± 0.01, which represents an increase of 40%.

Effect of amino acid probes on uptake into chromaffin granules

Table 5 demonstrates the effect of six amino acid probes on ATP and noradrenaline uptake into chromaffin granules. Pyridoxal phosphate inhibited both uptake processes to a similar degree. DIDS preferentially blocked ATP uptake, whereas phenylglyoxal only inhibited ATP transport.

Discussion

The first part of the present paper is concerned with the specificity of the carrier that transports nucleotides into chromaffin granules. This carrier does not only transport adenine nucleotides (ADP, ATP), uridine and guanosine triphosphate, as shown previously (Aberer *et al.*, 1978; Weber & Winkler, 1981), but also has an affinity for phosphoenolpyruvate, PO_4^{3-} and SO_4^{2-}. Thus, these compounds inhibit ATP uptake, but do not interfere with catecholamine transport, which is consistent with a competition for the nucleotide carrier. They are also taken up into chromaffin granules by a process that is inhibited by the same compounds that also interfere with ATP uptake. Thus inhibition occurs in the presence of atractyloside, a blocker at the carrier level (Aberer *et al.*, 1978), in the presence of uncouplers of oxidative phosphorylation, which abolish the electrochemical proton gradient (see Njus & Radda, 1978), and in the presence of SCN^-,

which neutralizes the electrical part of this gradient (Johnson & Scarpa, 1979).

Under our incubation conditions the uptake of catecholamines, PO_4^{3-}, phosphoenolpyruvate and SO_4^{2-} occurs also without the addition of exogenous ATP. All of these compounds, as shown previously (Aberer *et al.*, 1978) and in the present paper, depend for their transport on the electrochemical proton gradient. Apparently sufficient ATP released from the granules is present to drive these uptakes (Aberer *et al.*, 1978). However, a limited activation (77%) of catecholamine uptake was observed when Mg^{2+} (1 mM) and ATP (2 mM) were added (Weber & Winkler, 1981). In agreement, as shown here, addition of Mg^{2+} and ATP also activates $^{35}SO_4^{2-}$ uptake by 40%. The transport of this anion is dependent on $\Delta\psi$, since it is blocked by SCN^- as previously shown for ATP uptake (Weber & Winkler, 1981). However, these results do not demonstrate that this transport is an active uptake. In our studies with intact granules it cannot be excluded that SO_4^{2-} is taken up by an exchange with ATP that is present inside the vesicles in an extremely high concentration (0.15 M; see Winkler & Westhead, 1980). Studies on isolated granules ghosts (for catecholamine uptake, see Njus & Radda, 1978) are necessary to prove active uptake. In such ghost preparations the occurrence of concentration gradients from the inside to outside can be investigated.

The affinity of SO_4^{2-} for the carrier (apparent K_m, 0.4 mM) is similar to that of nucleotides (0.3–0.9 mM). On the other hand compounds containing sulphonic acid or carboxylic acid groups have no affinity for this carrier. This is also true for adenine and adenosine. Apparently, the carrier recognizes two strongly negative charged groups, as in sulphate or phosphate, whereas the nature of the compound connected with these charges has little influence.

Palmitoyl-CoA and Cibacron Blue were relatively unspecific in their effect on chromaffin granules. Both compounds did not only block nucleotide uptake but at least at the higher concentration tested inhibited also catecholamine transport. This might indicate that in addition to an effect on the nucleotide carrier these compounds also interfere with the ATPase providing the driving force for both uptakes. In mitochondria palmitoyl-CoA is an inhibitor of the nucleotide carrier (Chua & Shrago, 1977). Cibacron Blue is known to bind tightly to nucleotide-binding enzymes (see Edwards & Woody, 1979; Dean & Watson, 1979).

Our results on the broad specificity of the nucleotide carrier raise the question of the function of this carrier within the chromaffin cell. We have already pointed out previously (Weber & Winkler, 1981) that the presence of guanidine and uridine nucleotides within chromaffin granules (Goetz *et al.*, 1971) is consistent with a carrier transporting all these nucleotides. However, the carrier also transports sulphate, phosphoenolpyruvate and phosphate. For the first two molecules this has probably no physiological significance, since sulphate and phosphoenolpyruvate are not present in the cytosol in concentrations approaching the affinity of the carrier (approx. 0.5 mM). For phosphate this is likely to be different. For adrenal medulla the phosphate concentration in the cytosol is not known. However, in liver phosphate is present in a concentration similar to that of ATP, which is in the millimolar range (Bartels & Hohorst, 1963; Veech *et al.*, 1979). Thus one might expect that chromaffin granules *in vivo* accumulate phosphate ions and indeed Hillarp (1958) has shown that chromaffin granules do contain PO_4^{3-} (about one-fifth of the ATP concentration). Although Hillarp (1958) had already presented evidence to the contrary, this phosphate content was usually considered to have arisen from ATP breakdown during isolation and analysis. Since the nucleotide carrier also transports PO_4^{3-}, we can now confirm Hillarp's view that P_i is a genuine component of chromaffin granules. Apparently, catecholamines are stored together with phosphate compounds, which have a low permeability through the granule membranes. These compounds neutralize the positive charge of the catecholamines and may help in the storage of these amines (see Winkler & Westhead, 1980). For this purpose availability in the cytosol, low permeability through the granule membrane and strong negative charges are apparently the important criteria.

The nucleotide carrier also transports SO_4^{2-}. Phillips & Allison (1978) have already shown that chromaffin-granule ghosts accumulate this ion. However, the mechanism of uptake was not elucidated. If one wants to study ATP uptake into the chromaffin granule, interference from SO_4^{2-} has to be considered since $MgSO_4$ (instead of $MgCl_2$) is very often used for activating the ATPase. In such experiments catecholamine accumulation is not only accompanied by ATP uptake but also by that of SO_4^{2-}.

$^{35}SO_4^{2-}$ should prove to be a very suitable tool for studying the nucleotide carrier in various storage organelles. It can be obtained with very high specific activity, it has a very low diffusion component and it is economical. It would therefore be interesting to study SO_4^{2-} uptake, e.g. into cholinergic vesicles, where an ATP transport very similar to that of chromaffin granules has already been demonstrated (Luqmani, 1981).

In the second part of this paper we present the effects of amino acid probes on the uptake system into chromaffin granules. Pyridoxal phosphate reacting with NH_2 groups (Carraway, 1975) inhibited both uptake processes. This might be caused by an inhibition of both carriers, but more likely (see Pazoles *et al.*, 1980) it inhibits the ATPase. The same applies to 7-chloro-4-nitrobenzo-2-oxa-1,3-di-

azole, considered (Ting & Wang, 1980) to be specific for tyrosine residues, to *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, a carboxy group reagent (Block *et al.*, 1981) and also to 2-hydroxy-5-nitrobenzyl bromide, reacting with tryptophan residues (Horton & Koshland, 1965), although this reagent shows some preferential inhibition of catecholamine uptake. DIDS is more specific since it inhibits preferentially nucleotide transport. This indicates that functionally important amino groups, which are sensitive to this reagent (see Carraway, 1975), are present in the carrier. The specific inhibition of the nucleotide carrier by phenylglyoxal is the most interesting result. This reagent is said (see Cross, 1981) to be specific for arginine residues. Our results therefore indicate that the nucleotide carrier has arginine moieties in a crucial position. It is very interesting to note that the mitochondrial nucleotide carrier is also influenced by phenylglyoxal (Klingenberg & Appel, 1980) and that it has a characteristic group of three arginine residues near its *C*-terminal end (Babel *et al.*, 1981). One might therefore add a final word of speculation. The membrane of a transmitter-storing organelle may have been derived during cellular evolution from the mitochondrial inner membrane: both structures possess a proton-pumping ATPase. The F_1 subunits are biochemically (Apps & Schatz, 1979) and, as recently shown (Schmidt *et al.*, 1982), morphologically similar. The ATP/ADP-exchange carrier of mitochondria may have given rise to a nucleotide transporter. A vesicle containing such a proton pump (from the cytoplasmic side to the vesicle interior) and a nucleotide transporter would in fact represent a 'purinergic' storage organelle (Burnstock, 1981). With the acquisition by these vesicles of an amine carrier the specificity of the nucleotide carrier became of lesser importance and therefore it might have degenerated to a broad specificity quite different from the mitochondrial transporter. However, the crucial arginine centre of the mitochondrial carrier might have been maintained. Isolation of the granule carrier and a detailed comparison with the mitochondrial one (as has been achieved for the proton-pumping ATPase) might add weight to this speculation.

This work was supported by the Dr. Legerlotz-Stiftung and by the Fonds zur Förderung der wissenschaftlichen Forschung (Austria).

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