Role of ATP in the uptake of noradrenaline by membranes of adrenal chromaffin granules

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Isolated membranes of adrenal chromaffin granules take up monoamines, e.g. noradrenaline, dopamine and 5-hydroxytryptamine by an adenosine-5′-triphosphate (ATP)-dependent mechanism (Da Prada, Obrist, & Pletscher, 1975). Several findings indicate that splitting of ATP by membrane-bound, Mg-dependent ATPase (Hillarp, 1958) is necessary for this uptake (Da Prada & others, 1975; Kirshner, 1974; Taugner & Hasselbach, 1966). However, another possibility cannot be excluded, i.e. the function of ATP as a carrier or as part of a carrier system by which the amines are transported across the membranes, whereby ATP would not act as an energy source.

In order to distinguish between these two alternatives, experiments have been carried out with ATP analogues in which the oxygens linking the phosphorus atoms were substituted by CH_2 or NH groups. These nucleotide analogues possess structural features closely related to the naturally occurring polyphosphate groupings, but show resistance to cleavage at the point of methylene- or NH-substitution (Hecht & Sundaralingam, 1972; Larsen, Willett & Yount, 1969; Myers, Nakamura & Flesher, 1963; Yount, Babcock and others, 1971). Nucleotides with CH_2 or NH-substitutions in the side chain are generally accepted as tools in enzymology and membrane biology (Myers & others, 1963; Staehelin, Trachsel & others, 1975; Yount & others, 1971) and may also be used to establish whether ATP has a function as a carrier or as an energy source in the transport of biogenic amines through the membrane of the storage organelles.

In the present experiments, membranes of bovine adrenal chromaffin granules have been isolated as described by Da Prada & others (1975) and incubated in a microdiffusion chamber with various concentrations of ^{14}C-NA in the presence of either ATP or the α,β-methylene analogue (AMP-CPP), the β,γ-methylene analogue (AMP-PCP) or the β,γ-NH-analogue (AMP-PNP). The uptake of ^{14}C-NA was measured in a liquid scintillation counter (for details see Da Prada & others, 1975). In addition, the formation of inorganic phosphate during incubation of granule membranes with ATP or the three analogues was determined by a colorimetric method (Chen, Toribara & Warner, 1956).

As shown by Da Prada & others (1975), membranes incubated in the presence of ATP took up ^{14}C-NA, and the uptake increased with rising concentration of the amine reaching a saturation level. Upon replacement of the ATP with AMP-CPP, AMP-PCP or AMP-PNP, the ^{14}C-NA uptake was markedly diminished. The

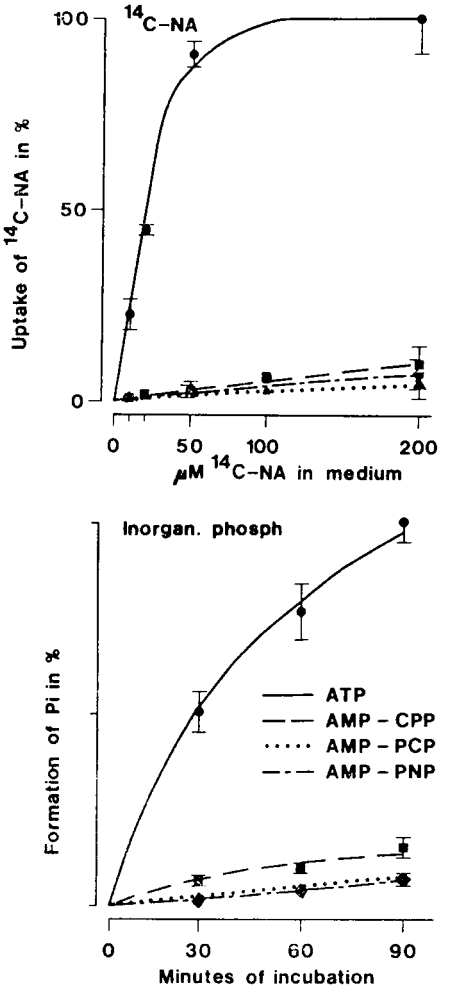
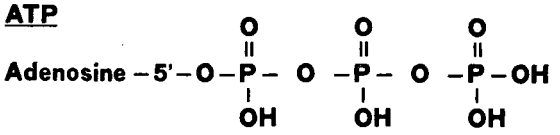


FIG. 1. Uptake of [^{14}C]noradrenaline (^{14}C-NA) and formation of inorganic phosphorus (Pi) by membranes of adrenal chromaffin granules in the presence of ATP and various analogues. The membranes were incubated (for 30 min in the uptake experiments) in 50mM Na-glycerophosphate buffer pH 7·4, containing 0·1 mM ascorbic acid, 5 mM $CaCl_2$ and 10 μM pargyline (*N*-benzyl-*N*-methyl-propargylamine, an inhibitor of monoamine oxidase) (Da Prada & others, 1975; Taugner, 1971). Initial concentration of ATP and analogues: 5 mM. Each point is an average with s.e.m. of 2–4 experiments. The values are expressed in % of those obtained with ATP and 200 μM ^{14}C-NA (above or with ATP at 90 min (below). Absolute values: uptake of ^{14}C-NA with ATP and 200 μM ^{14}C-NA in incubation medium (above): 94 ± 9 nmol mg^{-1} protein; formation of Pi after 90 min in the presence of ATP (below): 171 ± 9 nmol mg^{-1} protein.

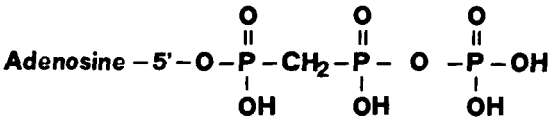
* Correspondence

ATP and analogues

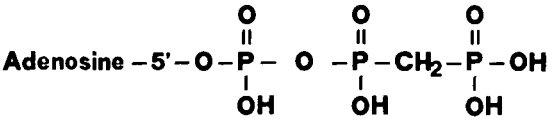
ATP



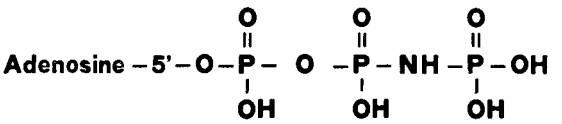
α,β-methylene-analogue, AMP-CPP



β,γ-methylene-analogue, AMP-PCP



β,γ-NH-analogue, AMP-PNP



values obtained with AMP-CPP were slightly, though not significantly ($P > 0.05$) higher than those with AMP-PCP and AMP-PCP (Fig. 1). Similarly, the formation of inorganic phosphate from ATP was much more marked compared with that from the analogues. Somewhat higher values were obtained with AMP-CPP than with AMP-PCP and AMP-PNP ($P < 0.01$ at 60 and 90 min) (Fig. 1).

These results indicate that the methylene- and NH-analogues, despite being similar to ATP in chemical structures, are much less susceptible to hydrolysis by membrane ATPase than ATP. The somewhat more marked degradation of AMP-CPP compared to AMP-PCP and AMP-PNP may be due to a partial splitting of the β,γ-P-O-P-bond of AMP-CPP which seems to be virtually impossible when the β,γ-oxygen is replaced by CH_2 or NH.

The presence of ATP as impurity in the AMP-CPP preparation which would explain the formation of inorganic P is unlikely because thin-layer chromatography of AMP-CPP (2-propanol-dimethylformamide-methylethylketone-H_2O-NH_4OH; 20:20:20:39:1) did not reveal detectable amounts of ATP (ATP content of AMP-CPP less than 1%).

Since the effect of ATP and its analogues in promoting ^{14}C-NA uptake by granule membranes differs markedly according to the susceptibility to degradation of these nucleotides by membrane ATPase and since adenosine-5′-diphosphate and adenosine-5′-monophosphate did not stimulate catecholamine uptake (Kirshner, 1962; unpublished results), the transport of ^{14}C-NA is likely to depend on energy liberated by hydrolysis of the nucleotides. The present results are similar to recent findings (Hoffman, Zinder & others, 1976) with isolated, intact bovine adrenal chromaffin granules containing high amounts of ATP and catecholamines. In this preparation AMP-CPP, AMP-PCP and AMP-PNP, in contrast to ATP, did not induce uptake of adrenaline.

It is concluded that in the uptake of monoamines by adrenal granule membranes ATP probably functions as an energy source and not as a carrier or part of a carrier system.

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REFERENCES

CHEN, P. S., TORIBARA, T. Y. & WARNER, H. (1956). *Analyt. Chem.*, **28**, 1756–1758.

DA PRADA, M., OBRIST, R. & PLETSCHER, A. (1975). *Br. J. Pharmac.*, **53**, 257–265.

HECHT, S. M. & SUNDARALINGAM (1972). *J. Am. chem. Soc.*, **94**, 4314–4319.

HILLARP, N.-Å. (1958), *Acta Physiol. Scand.*, **42**, 144–165.

HOFFMAN, P. G., ZINDER, O., NIKODIJEVIC, O. & POLLARD, H. (1976). *J. supramolec. Structure*, **4**, 181–184.

KIRSHNER, N. (1962). *J. biol. Chem.*, **237**, 2311–2317.

KIRSHNER, N. (1974). *Life Sci.*, **14**, 1153–1167.

LARSEN, M., WILLETT, R. & YOUNT, R. G. (1969). *Science*, **166**, 1510–1511.

MYERS, T. C., NAKAMURA, K. & FLESHER, J. W. (1963). *J. Am. chem. Soc.*, **85**, 3292–3295.

STAEHELIN, T., TRACHSEL, H., ERNI, B., BOSCHETTI, T. & SCHREIER, M. H. (1975). *Proc. 10th FEBS Meeting*, p. 309–323. Editors: Chapeville S. & Grunberg-Manago, Amsterdam, North Holland: New York: American Elsevier.

TAUGNER, G. (1971). *Arch. exp. Path. Pharmak.*, **270**, 392–406.

TAUGNER, G. & HASSELBACH, W. (1966). *Arch. Pharm. exp. Path.*, **255**, 266–286.

YOUNT, R. G., BABCOCK, D., BALLANTYNE, W. & OJOLA, D. (1971). *Biochemistry*, **10**, 2484–2489.