SHORT COMMUNICATION

Release of Catecholamines from Isolated Adrenal Chromaffin Granules by Endogenous ATP

A. M. POISNER[1] AND J. M. TRIFARÓ[2]

Department of Pharmacology, Albert Einstein College of Medicine, New York, New York 10461

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SUMMARY

By the use of ATP-regenerating systems it can be shown that the ATP leaking from adrenal chromaffin granules *in vitro* produces the same effects on these granules as does the addition of exogenous ATP, i.e., release of catecholamines and change in light-scattering properties.

We have demonstrated that the addition of ATP to isolated chromaffin granules causes the release of catecholamines, ATP, and protein in proportions comparable to those secreted from the intact adrenal gland in response to acetylcholine (1). This process was shown to be associated with structural changes as revealed by light-scattering changes in granule suspensions (2).

Since the chromaffin granules themselves are extremely rich sources of ATP, it would seem that the ATP leaking from chromaffin granules might also release catecholamines. However, the ATP escaping spontaneously from isolated chromaffin granules is ordinarily hydrolyzed to ADP and then to AMP by the granule membrane (3, 4), thus preventing the buildup of a high concentration of ATP in the suspension medium. By using several ATP-regenerating systems, we have now obtained evidence that endogenous ATP can act on chromaffin granules in the same way as exogenous ATP.

[1] Research Career Development Awardee, U.S.P.H.S.

[2] Senior Postdoctoral Fellow, U.S.P.H.S. Present address: Department of Pharmacology, McGill University, Montreal, Canada.

Preparation of chromaffin granules. Chromaffin granules were prepared from bovine adrenal medullae using Millipore filtration as described previously (1). The only modification was that vacuum filtration was employed rather than positive pressure generated by means of a syringe and barrel. This method reduces the yield of granules but also reduces the contamination by larger particles which can be squeezed through pores smaller than the particle size (S. Malamed, personal communication). For most studies granules filtered through 0.3 μ filters were used; for some, granules pushed through 0.22 μ filters were employed.

Incubation procedure. Incubations for studying catecholamine release were carried out at 30° as described before (1) in a standard incubation medium containing (mM): KCl, 160; NaCl, 5.0; $MgCl_2$, 0.5; PO_4 buffer, pH 7.0, 10. Incubations were terminated by rapidly diluting with ice-cold 0.3 M sucrose containing 2 mM EDTA, briefly cooling in an ice bath, and centrifuging at 20,000 *g* for 10 min. The supernatant was used to assay catecholamines (5).

Light-scattering experiments. Studies on light scattering were carried out using a Zeiss PMQ II spectrophotometer at 540 mμ

as described previously (2). Either 1.0 ml volumes plus 50 μl granule suspension or 2.0 ml volumes and 100 μl granules were employed.

Substrates and enzymes. ATP, creatine phosphate (CP), phosphoenolpyruvate (PEP), creatinephosphokinase (CPK), and pyruvate kinase (PK) were obtained from Boehringer Mannheim, New York. The CP and PEP were stated to be chromatographically free of adenine nucleotides. The combination of PEP plus PK and CP plus CPK will be referred to as the PEP-PK and CP-CPK systems, respectively.

Effect of ATP-regenerating systems on catecholamine release from isolated chromaffin granules. The PEP-PK and CP-CPK systems are known to catalyze the formation of ATP from ADP:

(1) PEP + ADP → ATP + pyruvate

(2) CP + ADP → ATP + creatine

When either PEP or CP (5 mM) was used by itself, a small and variable increase in catecholamine release was observed (lesser concentrations had no effect). The enzymes PK and CPK similarly had small or negligible effects. However, when the PEP was combined with PK, and CP was combined with CPK, significant catecholamine release was obtained which was clearly more than additive (Table 1). Furthermore, when the ATP-regenerating system was employed together with exogenous ATP, the releasing action of ATP was potentiated (Table 1).

In another series of experiments, the releasing effect of ATP + PEP + PK was compared on granules passed through 0.3 μ filters with granules passed through 0.22 μ filters. There was no difference between the two granule suspensions: The ATP + PEP + PK released 74.2 ± 1.0% of the granule catecholamines in 10 minutes using the 0.3 μ granules, and 77.8 ± 1.2% using the 0.22 μ granules.

Effect of ATP-regenerating systems on optical density of chromaffin granules suspensions. Previous work has shown that coincident with the release of catecholamines, ATP, and protein from chromaffin granules, there is a fall in optical density (OD) of granule suspensions (2). Therefore the effect of the PEP-PK and CP-CPK systems were examined using this light-scattering technique. Whereas PEP and PK alone had negligible effects on OD, together they caused a significant fall in OD. The effect of exogenous ATP was also enhanced in the presence of PEP-PK (Fig. 1). A similar result was obtained with the CP-CPK system: neither component had much

TABLE 1

Effect of ATP and ATP-regenerating systems on catecholamine release from isolated chromaffin granules

Isolated chromaffin granules were incubated at 30° in a medium containing (mM): KCl, 160; NaCl, 5; KPO_4 buffer, pH 7.0, 10; $MgCl_2$, 0.5. Incubation and preincubation times for each experiment are shown at the top. At the end of the preincubation period additions were made as follows: ATP (0.5 mM), PEP (5.0 mM), PK (1 μg/ml), CP (5.0 mM), CPK (1μg/ml). The total volume was 1.05 ml. Each value represents the percentage of bound catecholamines released above that of the control and is the mean (±SE) of three observations.

Experiment:	I	II	III	IV
Preincubation (min):	0	10	15	15
Incubation (min):	20	30	30	10
ATP	30.0 ± 1.0	49.7 ± 2.1	—	35.2 ± 0.3
PEP	—	14.8 ± 1.9	26.6 ± 4.5	—
PK	—	12.7 ± 1.1	−2.3 ± 1.3	—
PEP + PK	13.6 ± 0.7	41.8 ± 3.2	72.0 ± 5.5	—
ATP + PEP + PK	66.7 ± 0.2	—	—	—
CP	—	—	21.0 ± 1.4	3.3 ± 1.8
CPK	—	—	−0.1 ± 4.2	1.5 ± 0.1
CP + CPK	—	—	50.7 ± 2.5	19.8 ± 1.0

effect on OD by itself, but together they produced a marked fall in OD (Fig. 2).

Discussion. Many studies have shown that chromaffin granules can hydrolyze ATP to ADP, including the ATP which spontaneously leaks out from the granules. The present experiments (utilizing two independent ATP-regenerating systems) demonstrate that if this endogenous ATP is allowed to build up, then it can act on

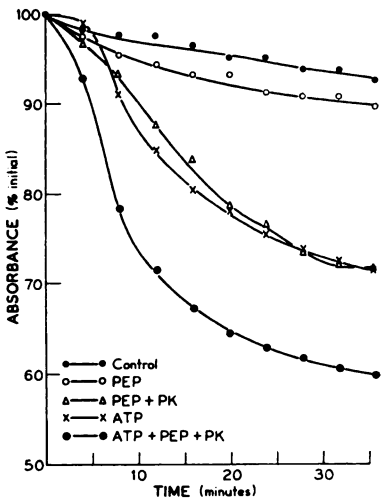

FIG. 1. *Effect of ATP, PEP, and PK on optical density of chromaffin granules*

Chromaffin granules were suspended in the standard medium. When additions were made at zero time, the final concentrations were: ATP, 0.5 mM; PEP, 5.0 mM, and PK, 1 μg/ml. Absorbance is expressed as a percentage of the value at zero time.

chromaffin granules in the same way as exogenous ATP. Both the phosphoenolpyruvate–pyruvatekinase and the creatinephosphate–creatinephosphokinase systems evoked release of catecholamines from chromaffin granules and produced the changes in optical density characteristic of release of soluble granule constituents (1, 2).

The chromaffin granules are by far the richest source of ATP in the chromaffin cell and therefore the most likely source of the ADP utilized by the PEP-PK and CP-CPK systems. However, since traces of mitochondria are present in 0.3 μ filtrates

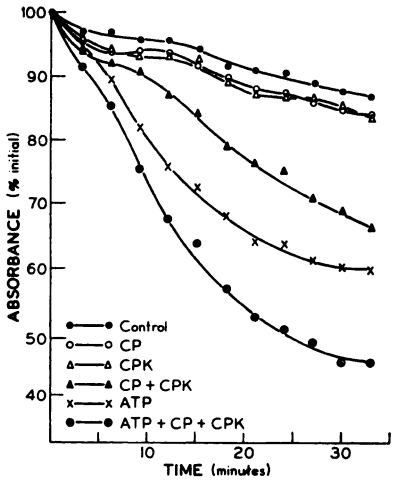

FIG. 2. *Effect of ATP, CP, and CPK on optical density of chromaffin granules*

Chromaffin granules were suspended in the standard medium. When additions were made at zero time the final concentrations were: ATP, 0.5 mM; CP, 5.0 mM; and CPK, 1 μg/ml. Absorbance is expressed as a percentage of the value at zero time.

of medullary homogenates, some experiments were done with 0.22 μ filtrates, where even less mitochondrial contamination is present (6). The effect of the PEP-PK system was the same with the two types of granule preparations and therefore must be due to the principal species present—the chromaffin granules.

The present results raise the question whether the spontaneous release of catecholamines observed in chromaffin granule preparations might be due to the action of endogenous ATP. Various workers have noted that the spontaneous release of catecholamines is greater in KCl media than in sucrose (7), and we have pointed out that sucrose inhibits the ATP-evoked release process (1). Since traces of Mg must have been present in these studies (Mg is required for the ATP-evoked release) the higher spontaneous release in KCl media than in sucrose may be partly accounted for by activation of the ATP-evoked release process.

We have suggested that ATP may be involved in the release of catecholamines from the adrenal gland *in vivo* in response

to acetylcholine (1). It is conceivable that the spontaneous release of catecholamines *in vivo* [which can be seen even in denervated glands (8)] may also be related to the action of ATP. Perhaps the nongranule ATPases and other ATP-utilizing systems, by limiting the concentration of endogenous ATP, are important factors in maintaining low levels of catecholamine secretion during resting conditions *in vivo*.

It can be calculated that the ATP levels *in vitro* achieved by leakage of granule ATP must be much less than 0.05 mM; and, since the PEP-PK and CP-CPK systems must be acting on ADP derived from granule ATP, then it is clear that when the ATP level is stabilized an extremely low concentration of ATP can act on chromaffin granules.

This extreme sensitivity plus chemical and morphological similarities between release of catecholamines from the adrenal medulla and of vasopressin from the neurohypophysis (9, 10) suggest that a similar process may be involved in the release of this neurosecretory substance. This possibility is now under investigation in our laboratory. It is hoped that further studies will reveal whether ATP and ATPase are involved in the release of other granule-bound substances from nerve endings and exocrine glands.

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REFERENCES

1. A. M. Poisner and J. M. Trifaró, *Mol. Pharmacol.* **3**, 561 (1967).
2. J. M. Trifaró and A. M. Poisner, *Mol. Pharmacol.* **3**, 572 (1967).
3. N.-Å Hillarp, *Acta Physiol. Scand.* **42**, 144 (1958).
4. A. D'Iorio, *Can. J. Biochem. Physiol.* **35**, 395 (1957).
5. A. H. Anton and D. F. Sayre, *J. Pharmacol. Exptl. Therap.* **138**, 360 (1960).
6. M. Oka, T. Ohuchi, H. Yoshida and R. Imaizumi, *Life Sci.* **5**, 427 (1966).
7. R. Greenberg and H. Sabelli, *Proc. Soc. Exptl. Biol. Med.* **116**, 705 (1964).
8. M. Vogt, *Brit. J. Pharmacol.* **7**, 325 (1952).
9. W. W. Douglas and A. M. Poisner, *J. Physiol. (London)* **172**, 1 (1964).
10. W. W. Douglas and A. M. Poisner, *J. Physiol. (London)* **172**, 19 (1964).