Biochemical Pharmacology, Vol. 22, pp. 335–339. Pergamon Press, 1973. Printed in Great Britain

THE ACTION OF ATP IN THE GUINEA-PIG HEART

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(*Received* 20 *April* 1972; *accepted* 22 *August* 1972)

Abstract—Experiments were designed to compare the potencies of ATP and adenosine in the isolated guinea-pig heart. Adenosine appeared to be twice as potent as ATP in slowing the heart; however, ATP was broken down to AMP and adenosine in the perfusion fluid. ATP was completely dephosphorylated to adenosine before uptake by the heart. It is concluded that breakdown of ATP to adenosine precedes pharmacological action in the guinea-pig heart *in situ*, where these compounds appear to be equipotent. The potency of ATP itself remains elusive; however, the results suggest that the adenyl phosphates are pharmacologically active.

ADENOSINE and its phosphorylated derivatives ATP, ADP and AMP, cause relaxation of smooth muscle and reduction in the rate and force of cardiac contractions in many species.[1–6] However, after administration of these nucleotides to animals or isolated organs, rapid enzymatic breakdown to lower phosphates or adenosine is likely to occur, and the extent to which this breakdown influences pharmacological activity is difficult to assess. In some organs, ATP is more potent than adenosine or AMP,[4–6] and this indicates that dephosphorylation of ATP is not necessarily a prerequisite for pharmacological activity, and may in fact reduce apparent potency.

In the guinea-pig heart *in situ*, equimolar doses of ATP, ADP, AMP and adenosine injected into the left atrium produce a period of heart block identical both in latency and in duration.[1] This could mean either that the four compounds are equipotent, or that dephosphorylation of the nucleotides occurs so rapidly that their true potency is not assessed.

Because much of the enzymatic dephosphorylation of the nucleotides under these conditions would probably occur in the blood, it seemed reasonable to compare the potencies of ATP, AMP and adenosine in the isolated heart perfused with salt solution, to see whether potency differences between the nucleotides and adenosine might be related to rapidity of dephosphorylation in the tissue.

METHODS

Guinea-pigs. Those used were of either sex and weighed between 250 and 300 g, the hearts of which weighed about 800 mg.

Potency comparisons. The potencies of adenosine and AMP were compared with ATP. Each guinea-pig heart was isolated and perfused through an aortic cannula with McEwen's[7] solution, saturated with 5% CO_2 in O_2. The temperature of the perfusion fluid was 32° and the flow rate was maintained at 6·5 ml/min by a Watson–Marlow peristaltic pump. Adenyl compounds were injected at 2 min intervals, in volumes of 0·2 ml and amounts of 1–20 nmoles, into the perfusion fluid at a point close to its entry into the heart. Spontaneous ventricular contractions were recorded with an

isotonic microdynamometer (Ugo Basile, Milan) writing on a smoked drum. Only two compounds were compared on a single heart, and were administered alternately.

Breakdown of ATP. The fate of injected ATP in the perfusion fluid was studied using [8-^{14}C]ATP. In one series of experiments [8-^{14}C]ATP (10 nmoles, 2 nCi) was injected into the perfusion fluid of each heart as described above, the perfusate being collected for 10 sec following the injection in a tube standing in ice. In another series of experiments [8-^{14}C]ATP (10 nmoles, 2 nCi) was added to samples of perfusion fluid prior to chilling. Extraction of the ^{14}C-labelled compounds from the perfusates was achieved using Norit A, from which recovery was about 80 per cent for each adenyl compound. Separation was carried out on Whatman chromatography paper (4 MM), measuring 30 × 20 cm. Carrier ATP, ADP, AMP, inosine and adenosine were included. Each chromatogram was run in two dimensions, firstly in isobutyric acid–H_2O–NH_3–0·1 M EDTA (100:46:14:1·6) pH 4·6, and secondly in 1 M ammonium acetate including 0·01 M EDTA–90% ethanol (30:70). After location of markers under u.v. light the paper in each of these areas was cut into 3 × 3 mm pieces and suspended in 0·4% 2,5-*bis*-2-(5-tertbutylbenzoxazolyl)-thiophene (Packard) in toluene to which had been added 4% Thixotropic-gel (Packard). The amount of ^{14}C-label in each sample was measured in a Packard Tricarb liquid scintillation spectrometer.

Uptake of ATP. This was studied by comparison of the retention of the radioactive label after injection of 10 nmoles of each [8-^{14}C]ATP (500 nCi), [α-^{32}P]ATP (7μCi), [γ-^{32}P] ATP (35 μCi), and [8-^{14}C]adenosine (280 nCi). The procedure was that described previously,[8] except that, in addition, the ^{32}P-labelled nucleotides were separated from ^{32}P-labelled inorganic phosphate chromatographically. Extracts were prepared as described above, aliquots of 0·1 ml were chromatographed on Whatman (4 MM) chromatography paper using di-isopropylether–formic acid–*n*–butanol (40:20:30) as solvent. Inorganic phosphate was detected by spraying with ammonium molybdate reagent.

Inhibition of adenosine uptake. It has previously been demonstrated that adenosine uptake in guinea-pig perfused hearts follows saturation kinetics.[8] This fact was used to determine whether ATP or its products of hydrolysis significantly inhibit the adenosine carrier. [8-^{14}C]adenosine (28 mCi/mmole) was used as substrate to yield the following concentrations: 2×10^{-6}, $6{\cdot}6 \times 10^{-7}$, 4×10^{-7} and $2{\cdot}9 \times 10^{-7}$ M. In hearts to which ATP was administered it was injected with the substrate to yield a concentration of 2×10^{-6} M. Subsequent procedures were as has been previously described.[8]

Materials. ATP, ADP, AMP, inosine and adenosine (Sigma), [8-^{14}C]ATP (50 mCi/mmole), [γ-^{32}P]ATP (13·2 Ci/mmole), [α-^{32}P]ATP (1·3 Ci/mmole) and [8-^{14}C]adenosine (28 mCi/mmole) (Radiochemical Centre, Amersham).

RESULTS

Potency comparisons. Responses of the guinea-pig isolated heart to adenosine, AMP and ATP were measured as percentage reductions in the rate of ventricular contractions. Log dose–response lines were plotted, and potency ratios were estimated on a molar basis. The potency of ATP relative to adenosine was 0·63 ± 0·07 (mean ±S.E. of 6 observations). ATP and AMP were found to be equipotent in 3 experiments. However, interpretation of these results depends on the extent of breakdown of ATP and AMP.

TABLE 1. CHEMICAL BREAKDOWN OF [8-^{14}C]ATP IN GUINEA-PIG PERFUSED HEART AND IN THE PERFUSATE

ATP	ADP	AMP	Adenosine	Inosine
(a) 4 ± 1	3 ± 1	80 ± 2	10 ± 2	2·4 ± 0·2
(b) 9 ± 3	33 ± 6	57 ± 9	0	0

[8-^{14}C]ATP (10 nmoles, 2 nCi) was (a) injected into the cannula of perfused guinea-pig heart, (b) placed in a catching vessel below a perfused guinea-pig heart. In both (a) and (b) perfusate was collected for 10 sec chilled and analysed for breakdown products of [8-^{14}C]ATP. Results are expressed as percentages of total ^{14}C-label. Each result is the mean, with S.E. of (a) 7 observations, (b) 3 observations.

Breakdown of ATP. ATP injected into the fluid perfusing the guinea-pig isolated heart was rapidly broken down. In the effluent fluid collected 10 sec after injection of ATP, the main breakdown product was AMP; some adenosine and inosine were also formed (Table 1).

Furthermore, rapid breakdown of ATP also occurred when it was added to samples of perfusate collected after passage through the heart, only about 10 per cent of the ATP being present after 10 sec (Table 1). This suggests that ATPases are easily leached out from the guinea-pig heart into the perfusion fluid; a similar observation has been made with adenosine deaminase in cat perfused heart.[9] It is therefore extremely difficult to assess accurately the degree of breakdown of ATP in the coronary circulation and especially in the region of the cardiac receptors.

Uptake of ATP. After a single injection of 10 nmoles of [8-^{14}C]ATP into the guinea-pig isolated heart, about 10 per cent of the radioactive label was retained within the heart. Uptake of the adenosine moiety from ATP was only one third of the uptake after the same dose of adenosine (Table 2).

Uptakes of the ^{32}P labelled α- and γ-phosphate moieties from ATP were only 7 and 13 per cent respectively, of the uptake of the ^{14}C labelled adenosine moiety.

TABLE 2. UPTAKE OF THE RADIOACTIVE LABEL BY ISOLATED GUINEA-PIG HEART AFTER PERFUSION WITH 10 nmoles OF LABELLED ATP OR ADENOSINE

	Uptake	Label in P_i
[8-^{14}C]ATP	1·16 ± 0·13	
[α-^{32}P]ATP	0·078 ± 0·013	0·059 ± 0·007
[γ-^{32}P]ATP	0·148 ± 0·018	0·119 ± 0·020
[8-^{14}C]Adenosine	3·0 ± 0·3	

ATP (8-^{14}C 500 nCi, γ-^{32}P 7 μCi, α-^{32}P 35 μCi) or [8-^{14}C] adenosine (280 nCi) was injected into the perfusion fluid over a period of 1 min. Each heart weighed about 800 mg. One min later, each heart was removed from its cannula and prepared for analysis. Each result is the mean expressed as nmoles/g, with S.E. of four observations.

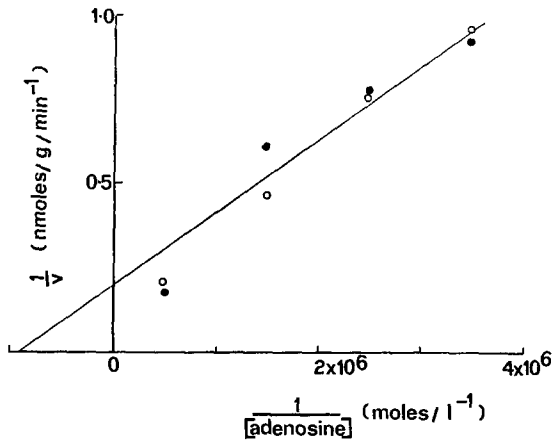

FIG. 1. A double reciprocal plot of the uptake of [8-^{14}C]adenosine by perfused guinea-pig hearts in the absence (●——●) and in the presence (○——○) of 2×10^{-6} M ATP.

Moreover, the major portion of the ^{32}P retained by the heart was in the form of inorganic phosphates. These results show that ATP is virtually completely hydrolysed to adenosine before being taken up by the guinea-pig heart.

Inhibition of adenosine uptake. It was found that 2×10^{-6} M ATP did not affect the rate of incorporation of adenosine by the heart (Fig. 1).

DISCUSSION

In the guinea-pig isolated heart, ATP and AMP were only about 60 per cent as potent as adenosine, whereas in the guinea-pig heart *in situ*,[1] all three compounds were equipotent. It therefore appears that, in the whole animal, complete dephosphorylation of the nucleotides to adenosine occurs before they reach their site of action, and they appear to be equipotent with adenosine merely because they are acting as adenosine. In the isolated heart perfused with McEwen's[7] solution, dephosphorylation is less complete, probably because of the absence of blood. Nevertheless, even in the isolated heart, appreciable dephosphorylation of ATP and AMP is likely to take place before they reach their site of action, and both compounds are probably acting on cardiac receptors as a mixture of AMP and adenosine. Therefore no assessment of the true potency of ATP is possible under these conditions.

Experiments with [8-^{14}C]ATP and with ^{32}P-labelled ATP, demonstrated that virtually all the phosphate groups are split off ATP before the remaining adenosine moiety is taken up by the guinea-pig heart. Because the ^{14}C-label was in the purine ring, these experiments did not prove conclusively that the moiety taken up by the heart was adenosine, and not adenine. However, Kolassa *et al.*[10] demonstrated that the uptake of adenine by the guinea-pig isolated heart is considerably less than the uptake of adenosine. It therefore seems reasonably certain that it is adenosine that is taken up after the injection of ATP. Because the β- and γ-phosphate groups of ATP are hydrolysed more readily than the α-phosphate less of the α-^{32}P than the γ-^{32}P appears as available inorganic phosphate. This accounts for the lower levels of α-^{32}P in the heart (Table 2). Previous work from this laboratory[11] has shown that [8-^{14}C]

adenosine taken up by the guinea-pig isolated heart is rapidly converted to ATP, and retained in that form. Thus the fate of ATP after injection into the guinea-pig heart is that it is rapidly broken down to a mixture comprising mainly AMP and adenosine; much of the adenosine is then taken up into the heart where it is reconstituted with different phosphate groups back into ATP.

Some idea of the amount of ATP which was broken down to adenosine can be gained from the results of uptake studies. Uptake of adenosine formed from the breakdown of 10 nmoles of ATP was only one-third the uptake after injection of 10 nmoles of adenosine. On the basis of a Michaelis constant of $1{\cdot}0 \times 10^{-6}$ M and a velocity constant 4·5 nmoles/g/min for the uptake of adenosine,[8] it is calculated that 25 per cent of the injected ATP formed adenosine in the coronary vessels. Such a calculation is valid only where there is no inhibition of the uptake process by other breakdown products. With a concentration of ATP which would have yielded negligible inhibition of adenosine uptake by formation of competing substrate, no inhibition was observed. A 25 per cent breakdown to adenosine was greater than that seen in the fluid from perfused hearts. This suggests dephosphorylation of ATP to AMP occurs in the coronary circulation as a whole, but breakdown to adenosine is more localized to the smaller vessels or the extracellular space. In support of this idea there was no evidence that 5′-nucleotidase leached into the perfusate. Histochemical studies in heart have demonstrated that the enzyme is in close association with the sarcolemma.[12] This enzyme may be rate-limiting in the formation of adenosine. A lack of difference in potency of ATP and AMP is in keeping with the presence of a rate-limiting step, such that the amounts of adenosine produced are independent of the concentration of AMP.

It can be estimated from the potencies of ATP and AMP that the adenyl-phosphates themselves contributed to the pharmacological action, if it is assumed that only 25 per cent of the mixture in the region of the receptor was adenosine. However, no assessment of the potency of ATP itself can be made from these results.

Acknowledgement—The author thanks Dr. Anne Stafford for her helpful advice during this study.

REFERENCES

1. A. Stafford, *Br. J. Pharmac.* **28,** 218 (1966).
2. T. N. James, *J. Pharmac. exp. Ther.* **149,** 233 (1965).
3. A. Versprille, *Pflügers Arch.* **291,** 261 (1966).
4. J. Axelsson and B. Holmberg, *Acta physiol. Scand.* **75,** 149 (1969).
5. H. A. Kontes, D. Goldin, D. W. Richardson and J. L. Patterson, *Am. J. Physiol.* **214,** 107 (1968).
6. G. Burnstock, G. Campbell, D. Satchell and A. Smythe, *Br. J. Pharmac.* **40,** 668 (1970).
7. L. M. McEwen, *J. Physiol., Lond.* **131,** 678 (1956).
8. S. V. Hopkins and R. G. Goldie, *Biochem. Pharmac.* **20,** 3359 (1971).
9. M. I. Jacob and R. M. Berne, *Am. J. Physiol.* **198,** 322 (1960).
10. N. Kolassa, K. Pfleger and W. Rummel, *Eur. J. Pharmac.* **9,** 265 (1970).
11. S. V. Hopkins, *Biochem. Pharmac.* in press.
12. G. B. Gordon, H. M. Price and J. M. Blumberg, *Lab. Invest.* **16,** 422 (1967).