Pflügers Arch. 367, 129–135 (1976)

Pflügers Archiv
European Journal of Physiology

Compartmentation of Cardiac Adenine Nucleotides and Formation of Adenosine*

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Summary. After prelabeling the adenine nucleotides (ATP, ADP, AMP) of isolated perfused guinea pig hearts with either ^{14}C-adenine or ^{14}C-adenosine for 35 min, labeled adenosine, inosine, hypoxanthine and cyclic 3′5′-AMP (cAMP) were continuously released into the cardiac perfusate. Determination of the specific activities (SA) of the adenine nucleotides, cAMP, and their breakdown products (adenosine, inosine, hypoxanthine) in tissue and perfusate revealed: Under steady state conditions the SA of adenosine and cAMP in the perfusate were of the same order of magnitude and proved to be many times higher than the SA of the respective precursor adenine nucleotides. This difference was observed regardless whether adenine or adenosine was used as prelabeling substance. The SA of inosine and hypoxanthine in the perfusate were constantly lower than the SA of adenosine. Cardiac ischemia of 6 min, which resulted in a markedly increased formation of adenosine, led to a pronounced decrease in the SA of adenosine released from the heart.

Our findings provide evidence that at least two different adenine nucleotide compartments of the heart serve as precursors for the formation of adenosine and cAMP, one characterized by a high, the other by a lower SA. Under normoxic conditions adenosine and cAMP released into the cardiac perfusate are derived mainly from a nucleotide fraction of high SA, which appears to be rather small. During ischemia a second compartment of much lower SA in addition contributes to the formation of adenosine.

Key words: ^{14}C-adenine – Salvage of purine nucleosides and bases – Isolated perfused guinea pig heart – Cyclic 3′5′-AMP – Inosine – Hypoxanthine.

INTRODUCTION

The metabolism of adenosine in the myocardium and its functional importance have been the subject of numerous investigations, mainly because of the proposed role of this vasoactive nucleoside in the metabolic regulation of coronary flow [2, 16]. Based on cell fractionation and histochemical studies [17], it has been postulated that adenosine, a degradation product of the adenine nucleotides, is formed in the myocardium at or within cell membranes lining compartments open to the extracellular space. However, no information seems to be available as yet as to whether the cardiac adenine nucleotides as a whole are involved in the formation and release of adenosine or whether a compartmentalized fraction of these nucleotides serves as precursor.

In an attempt to contribute to the elucidation of this problem the adenine nucleotides of isolated perfused guinea pig hearts were labeled with ^{14}C-adenine or ^{14}C-adenosine. Subsequently the specific activities of the nucleotide fractions and their degradatives (adenosine, inosine, hypoxanthine) as well as of cyclic 3′5′-AMP (cAMP) were determined in tissue and perfusate. The data obtained under different experimental conditions make it possible to draw some conclusions concerning functional compartmentation and precursor product relationship of the different purine compounds in the heart.

MATERIALS AND METHODS

The enzymes adenosine deaminase (EC 3.5.4.4), nucleoside phosphorylase (EC 2.4.2.1), xanthine oxidase (EC 1.2.3.2), phospho-

* A preliminary report of part of this work appeared in "Biochemistry and Pharmacology of Myocardial Hypertrophy, Hypoxia and Infarction" Vol. 7 of "Recent advances in studies on cardiac structure and metabolism". (P. Harris, R. J. Bing, A. Fleckenstein, eds.), pp. 171–175. München: Urban & Schwarzenberg 1976

This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft (Ge 129/8)

diesterase (EC 3.1.4.17) and the materials for the protein binding assay of cAMP were purchased from Boehringer Mannheim. (8-^{14}C)-adenine (62 mCi/mmol), (U-^{14}C)-adenine (282 mCi/mmol) and (U-^{14}C)-adenosine (587 mCi/mmol) were obtained from Amersham Buchler, Braunschweig. Radiochemical purity of these compounds as determined by thin-layer chromatography (TLC) on silica-gel (solvent system given below) was at least 98%. Silica-gel TLC plates (SIL G/UV_{254}) were purchased from Macherey und Nagel, Düren. PEI-cellulose (Servacel) was obtained from Serva, Heidelberg. Activated charcoal (Aktivkohle, No. 2186) and all other analytical reagent grade chemicals were purchased from Merck, Darmstadt.

Animal Experiments

Guinea pigs (Pirbright white) weighing 250–300 g were stunned by a blow to the head. The hearts were rapidly excised and rinsed with ice cold saline. Retrograde aortic perfusion according to the Langendorff technique (perfusion pressure: 60 cm H_2O) was immediately initiated. In all experiments a non-recirculating modified Krebs-Henseleit solution [3] (NaCl 116 mM, KCl 4.7 mM, $MgSO_4$ 1.1 mM, KH_2PO_4 1.17 mM, $NaHCO_3$ 24.9 mM, $CaCl_2$ 2.52 mM, glucose 8.32 mM, pyruvate 2.0 mM), equilibrated with 95% O_2 – 5% CO_2, 37° C, pH 7.4, was used. To assure that the isolated hearts were non-working preparations, the mitral valve was cut and thereby made insufficient. Following a 30 min period of equilibration the hearts were perfused for 35 min with ^{14}C-adenine (adenine concentrations: 0.04, 0.42 and 10 μM, respectively) or ^{14}C-adenosine (adenosine concentration: 0.4 μM). In the experiments with adenine, perfusion pressure was kept constant (60 cm H_2O). During prelabeling with adenosine, constant flow perfusion (Masterflex-pump) was applied (flow 8–9 ml/min; perfusion pressure > 40 cm H_2O).

In special experiments cardiac ischemia was induced by occluding aortic inflow of hearts which had been prelabeled with (8-^{14}C)-adenine (0.42 μM) for 35 min.

Coronary perfusates were collected under continuous stirring in beakers, which contained 10 ml of 1 N perchloric acid and were placed in an ice bath. The acidified perfusate was then treated with activated charcoal as described below.

Analytical Procedures

Tissue Extraction. After appropriate periods of perfusion, the hearts still attached to the perfusing system, were quickly frozen between aluminum blocks pre-cooled in liquid nitrogen. Connective tissue, atria and frozen perfusate were removed with a dental drill under liquid nitrogen. Then the ventricles were ground to a fine powder and extracted with 0.5 N perchloric acid (10 ml/g tissue). Following centrifugation the extract was neutralized (pH 7.0) with KOH (10 N, 1 N, 0.1 N), kept in an ice bath for 30 min, and separated from the $KClO_4$ precipitate. Then the extract was freeze-dried and the residue taken up in 4 ml of water (0° C). This solution was stored at −18° C until analyses were carried out.

Analysis of ATP, ADP and AMP. A small portion of the extract (0.1–0.4 ml) was used for separation of adenine nucleotides in duplicate by paper chromatography according to methods described earlier [6]. In one chromatogram, quantitation of the different compounds was done by phosphorus assay. For determination of the radioactivities, the areas of the second paper chromatogram containing ATP, ADP and AMP (visually localized under ultraviolet light) were cut out and transferred into scintillation vials.

Analysis of Adenosine, Inosine, Hypoxanthine and cAMP. 20 mg of activated charcoal were added to an aliquot (3ml) of the tissue extract as well as to the total perfusate collected (20–180 ml). These suspensions were shaken for 1 h in the cold (4° C) in order to adsorb nucleotides, nucleosides and purine bases. The charcoal was then separated by centrifugation and washed three times with 40 ml of ice cold distilled water. Elution of all purine compounds adsorbed to the charcoal was performed by shaking twice for 1 h with 10 ml of pyridine (10%) in ethanol (50%) [22]. The combined eluates were evaporated to dryness, the residue taken up in 0.4 ml of water, an aliquot of which was then applied as streaks (8 cm) on thin-layer plates (silica-gel). The plates were developed twice in the same direction with *n*-butanol–ethyl acetate–methanol–ammonium hydroxide (25%) (7:4:3:4, v/v) [21]. Measured R_f-values were: adenine 0.74, adenosine 0.66, hypoxanthine 0.48, cAMP 0.40, inosine 0.33, AMP, ADP, ATP 0–0.08.

For quantitation, the areas of the thin-layer plates containing adenosine, inosine hypoxanthine and cAMP were scraped off. Inosine, hypoxanthine and cAMP were eluted together, whereas adenosine was eluted alone [2 × 5 ml ethanol (50%)]. After evaporation of the eluates each residue was dissolved in 1.4 ml sodium phosphate buffer (50 mM, pH 7.4) and filtered (Millipore MF, 0.22 μm pore size). Analyses of inosine, hypoxanthine and adenosine were done after stepwise enzymatic degradation of each compound to uric acid by means of a dual-wavelength, double-beam spectrophotometer (Perkin Elmer, model 356) [10, 11, 14]. The limit of quantitation of each purine compound was 50 pmoles. Analysis of cAMP was performed according to the radioisotope dilution assay of Gilman [7] in an aliquot of the dissolved residue of the respective TLC eluate. In some experiments cAMP was quantitated after its separation by chromatography on PEI-cellulose columns from other purines present in the charcoal eluate [19]. Almost identical values were obtained by this procedure.

For determination of the radioactivities of adenosine, inosine, hypoxanthine and cAMP, an aliquot of the charcoal eluates, to which carrier amounts (80 nmoles) of the respective purines had been added were separated by thin-layer chromatography, as described above. The purine containing areas of the plates were individually scraped off and eluted twice with 5 ml of 50% ethanol. The combined eluates were then transferred into scintillation vials, evaporated to dryness and taken up in 1 ml H_2O, before addition of 10 ml of scintillation fluid.

Recovery Studies. In order to determine recovery rates of the different purines 0.2 nmoles of radioactive adenosine, inosine, hypoxanthine and cAMP, respectively, were added to perfusates (collected for 30 min) and to acid tissue extracts. Total recovery ranged for all compounds between 79 and 82%. Reported data (nmoles/g tissue or pmoles/ml perfusate) are not corrected for losses incurred during the different steps of analysis.

Radioactivity Measurements. Radioactivities were measured in a Packard tricarb liquid scintillation spectrometer (model 3380). Each vial contained 10 ml scintillation fluid (0.1 g POPOP, 5.5 g POP, 750 ml toluene, 250 ml triton X-100). Counting efficiencies for ^{14}C ranged from 85–90% under these conditions.

Reported relative specific activities were calculated by relating the specific activity of each purine compound to the specific activity of the precursor adenine or adenosine in the perfusion medium.

The radiopurity of adenosine, inosine and cAMP after chromatographic separation on TLC was assessed by specific enzymatic degradation of each of these compounds followed by chromatography of the degradatives. By this procedure the purines proved to be more than 92% radiochemically pure. In some experiments the fractions of the TLC eluate containing adenosine and cAMP, respectively, where precipitated three times with $ZnSO_4$ (0.2 M) and $Ba(OH)_2$ (0.2 M) [21]. This method yielded recoveries ranging between 90–95%.

Calculations. Based on the specific activity of adenine or adenosine in the perfusion medium (SA*), and the total radioactivity of the

myocardial adenine nucleotides (TA), the rate of incorporation of adenine and adenosine was calculated according to the following formula:

$$\text{Incorporation (nmoles/g/min)} = \frac{\text{TA (cpm} \times \text{g}^{-1} \times \text{min}^{-1})}{\text{SA* (cpm} \times \text{nmol}^{-1})}$$

RESULTS

In Table 1 data are compiled concerning tissue content of adenine nucleotides, cAMP, adenosine, inosine and hypoxanthine as well as the concentrations of cAMP, adenosine and its degradatives in the cardiac perfusate. Although the hearts were perfused with an artificial medium, the values for adenine nucleotides were similar to those reported for the heart in situ [4]. The tissue content of cAMP, adenosine, inosine and hypoxanthine was very low, comprising together only 0.1 % of the adenine nucleotides. Rather small amounts of cAMP, adenosine and its degradatives were present in the perfusate. The total loss of purines from the isolated guinea pig heart proved to be 22 nmoles/g/h, a value similar to the rate of synthesis de novo of myocardial adenine nucleotides in rat hearts [26].

Figure 1 demonstrates results of experiments in which the rates of release of ^{14}C-labeled adenine nucleotide degradatives and of cAMP were determined after prelabeling the hearts for 35 min with ^{14}C-adenine (adenine concentration: 0.42 μM). As can be seen, labeled adenosine, inosine, hypoxanthine and cAMP were released into the cardiac perfusate at almost constant rates over the experimental period of 80 min. Furthermore, it appears from Figure 1 that ^{14}C-adenine was also continuously liberated from isolated hearts. The initially greater release of this compound can be attributed mainly to the washout of ^{14}C-adenine which was retained during the prelabeling period. A similar pattern of release of radioactive purines was observed when the hearts were prelabeled with ^{14}C-adenine at different adenine concentrations (Table 2). Under these conditions tissue levels of adenine nucleotides, perfusate concentrations of the purines, coronary flow and heart rate, were not significantly altered.

Data concerning relative specific activities (RSA) of the various purine compounds in the myocardium as well as in the perfusate after prelabeling with ^{14}C-adenine at three different adenine concentrations are summarized in Table 2. Two findings deserve attention: (i) In all experiments RSA values of cAMP, adenosine, inosine and hypoxanthine in tissue and perfusate were markedly higher than the corresponding figures of the precursor nucleotides (ATP, ADP,

Table 1. Tissue content and perfusate concentrations of adenine nucleotides, cAMP, adenosine, inosine and hypoxanthine of the isolated perfused guinea pig heart under normoxic conditions. Myocardial tissue was analyzed after 60 and 90 min of perfusion; perfusate was collected for analyses between 60 and 90 min of perfusion. Mean values ± S.E.M.

	Myocardium nmoles/g	Perfusate pmoles/ml
ATP	3941 ± 18 (n = 32)	[a]
ADP	808 ± 41 (n = 31)	[a]
AMP	126 ± 6 (n = 33)	[a]
cAMP	0.32 ± 0.02 (n = 17)	0.5 ± 0.1 (n = 17)
Adenosine	2.13 ± 0.14 (n = 30)	4.0 ± 0.3 (n = 30)
Inosine	0.98 ± 0.07 (n = 28)	50.4 ± 4.7 (n = 30)
Hypoxanthine	1.26 ± 0.13 (n = 27)	10.0 ± 1.2 (n = 30)

[a] Not detectable

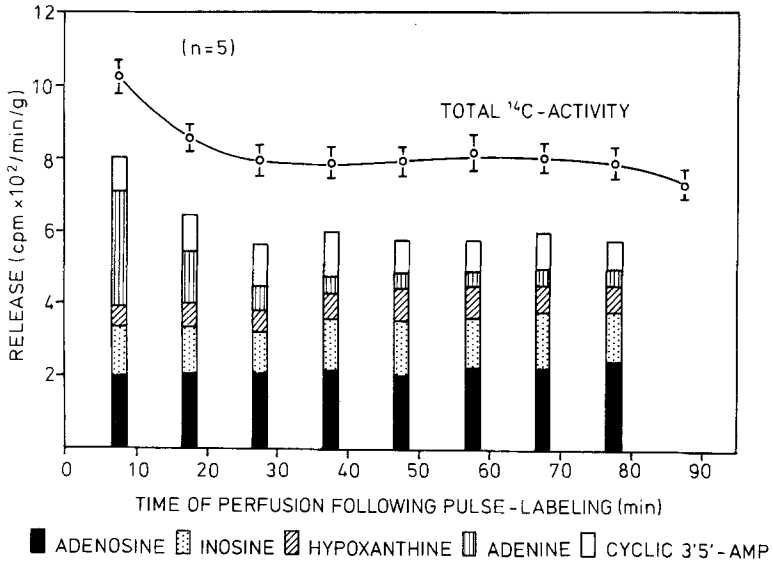

Fig. 1
Release of labeled adenosine, inosine, hypoxanthine, cAMP and adenine into the coronary effluent following prelabeling of the heart with ^{14}C-adenine for 35 min. Total ^{14}C-activity was measured directly in aliquots of the perfusate; the radioactivity of the purine compounds was estimated after their chromatographic separation (for details see Methods). Mean value ± S.E.M.

Table 2. Relative specific activities (RSA $\times 10^{-3}$) of adenine nucleotides, cAMP, adenosine, inosine and hypoxanthine in the myocardium and perfusate after 35 min prelabeling with ^{14}C-adenine at different concentrations of adenine. Myocardial tissue was analyzed at the end of the prelabeling period, whereas specific activities in the perfusate were determined after collection for 30 min following the prelabeling period. Mean values ± S.D.

Adenine concentration	0.04 µM		0.42 µM		10.0 µM	
Specific activity of adenine (CPM $\times 10^4$/nmole)[b]	66.0		13.5		2.6	
	Myocardium ($n = 5$)	Perfusate ($n = 3$)	Myocardium ($n = 4-7$)	Perfusate ($n = 9$)	Myocardium ($n = 4$)	Perfusate ($n = 4$)
ATP	0.11 ± 0.01	[a]	1.46 ± 0.17	[a]	5.43 ± 0.81	[a]
ADP	0.09 ± 0.03	[a]	0.98 ± 0.09	[a]	4.38 ± 1.48	[a]
AMP	0.07 ± 0.02	[a]	0.31 ± 0.13	[a]	3.47 ± 0.82	[a]
cAMP	0.64 ± 0.27	3.50 ± 0.99	3.20 ± 0.75	49.7 ± 11.3	31.80 ± 1.30	179.8 ± 16.2
Adenosine	0.28 ± 0.07	2.66 ± 0.36	3.28 ± 0.84	54.6 ± 21.9	30.80 ± 6.20	131.8 ± 21.1
Inosine	0.48 ± 0.05	0.19 ± 0.02	5.41 ± 1.66	3.3 ± 1.7	28.10 ± 15.10	21.7 ± 7.0
Hypoxanthine	0.61 ± 0.09	0.40 ± 0.18	2.23 ± 1.24	4.1 ± 3.1	21.10 ± 12.90	25.9 ± 8.5

[a] Not detectable
[b] (U-^{14}C)-adenine was used at adenine concentrations of 0.04 and 10.0 µM, (8-^{14}C)-adenine at the concentration of 0.42 µM

Table 3. Relative specific activities (RSA $\times 10^{-3}$) of adenine nucleotides, cAMP, adenosine, inosine and hypoxanthine in the myocardium and perfusate following volume constant perfusion for 35 min with (U-^{14}C)-adenosine (adenosine concentration 0.4 µM; specific activity of adenosine 15.4 CPM $\times 10^4$/nmole). Mean values ± S.D.

	Myocardium ($n = 5$)	Perfusate ($n = 4$)
ATP	3.91 ± 0.44	[a]
ADP	3.99 ± 0.26	[a]
AMP	3.92 ± 0.99	[a]
cAMP	12.40 ± 2.19	147.65 ± 34.78
Adenosine	9.66 ± 2.45	95.26 ± 35.31
Inosine	12.12 ± 2.36	17.62 ± 10.62
Hypoxanthine	16.33 ± 8.38	17.95 ± 6.07

[a] Not detectable

AMP). The RSA values of the adenine nucleotides were always in the order ATP > ADP > AMP. (ii) Among all compounds analyzed cAMP and adenosine in the perfusate exhibited the highest specific activities. Their RSA values exceeded by about one order of magnitude those of inosine and hypoxanthine in the perfusate and of cAMP, adenosine and hypoxanthine in the myocardium.

From the data in Table 2 rates of adenine incorporation into myocardial adenine nucleotides were calculated to be 0.9, 10.6 and 45.4 nmoles/g/h at adenine concentrations during the prelabeling period of 0.04, 0.42 and 10.0 µM, respectively. AV differences for adenine were measured in separate experiments. At the three different adenine concentrations used, only 13.7, 10.4 and 4.8 %, respectively, of adenine was taken up by the heart during a single passage.

A separate series of experiments was performed with ^{14}C-adenosine as prelabeling substance. At an adenosine concentration of 0.4 µM the uptake of this nucleoside proved to be 57 %. The rate of adenosine incorporation into adenine nucleotides amounted to 34 nmoles/g/h, a value three times higher than that for adenine incorporation at a comparable concentration. In Table 3, RSA values for all purine compounds in tissue and perfusate from hearts prelabeled with ^{14}C-adenosine are summarized. It is of particular interest that under these conditions, as well, by far the highest RSA values were found in the cAMP and adenosine fraction of the perfusate. Furthermore, the RSA values of cAMP, inosine and hypoxanthine also exceeded those of ATP, ADP and AMP. The pattern of the RSA values for all other compounds was similar to that observed in the experiments with adenine, except that the specific activities of ATP, ADP and AMP proved to be almost identical with adenosine as prelabeling substance.

In a further experimental series the effect of cardiac ischemia on the concentration and radioactivity pattern of the different purines in myocardium and perfusate was investigated. From the quantitative data given in Table 4 it appears that after coronary occlusion of 6 min, myocardial ATP was substantially diminished, while ADP, AMP, adenosine, inosine and hypoxanthine increased considerably. Tissue content of cAMP did not change significantly. In Figure 2, RSA values of the different purine compounds determined in cardiac tissue and perfusate after 6 min of

Table 4. Tissue content and perfusate concentrations (nmoles/g and pmoles/ml, respectively) of adenine nucleotides, cAMP, adenosine, inosine and hypoxanthine in controls under normoxic conditions and after 6 min of cardiac ischemia. In the latter experiments tissue was analyzed at the end of the ischemic period, whereas perfusate analysis was performed in samples collected for 5 min following release of ischemia. Mean values ± S.E.M.

	Myocardium		Perfusate	
	Control (n = 5)	Ischemia (n = 3)	Control (n = 5)	Ischemia (n = 3)
ATP	3998 ± 124	3113 ± 13	[a]	[a]
ADP	1013 ± 52	1432 ± 108	[a]	[a]
AMP	107 ± 11	305 ± 7	[a]	[a]
cAMP	0.32 ± 0.02	0.26 ± 0.05	0.4 ± 0.1	[b]
Adenosine	2.42 ± 0.89	17 ± 1.8	4.2 ± 0.9	51 ± 8
Inosine	0.92 ± 0.29	113 ± 11.8	62.7 ± 21.0	3957 ± 1222
Hypoxanthine	1.00 ± 0.42	61 ± 5.2	15.8 ± 9.8	718 ± 178

[a] Not detectable

[b] The amount of cAMP released into the perfusate during a 5 min period of collection was below the detection limit of our assay

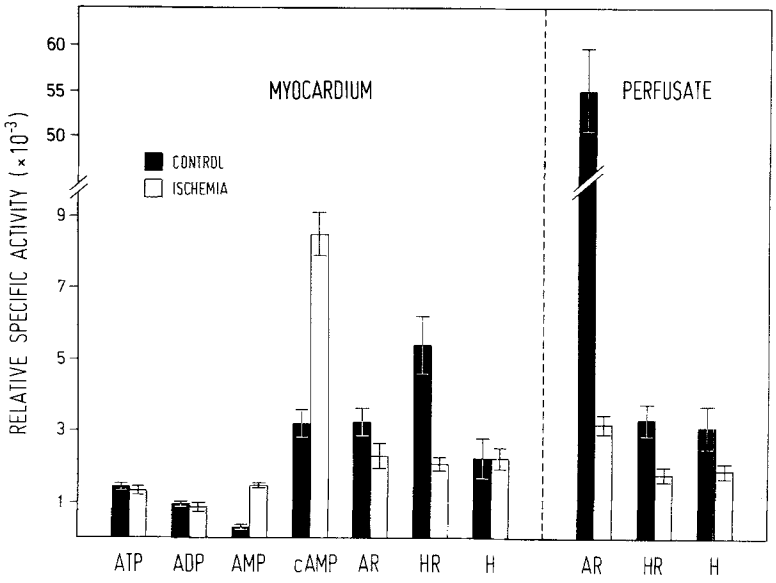

Fig. 2
Relative specific activities of adenine nucleotides, cAMP, adenosine (*AR*), inosine (*HR*) and hypoxanthine (*H*) in controls and after 6 min of cardiac ischemia. The hearts were prelabeled with ^{14}C-adenine (0.42 µM) for 35 min. Analyses and radioactivity measurements in tissue and pefusate were performed according to the protocol given in Table 4. Values for normoxic hearts ("control") were taken from Table 2. Values for the ischemic myocardium and for perfusate collected after release of ischemia represent three experiments each. Mean value ± S.E.M.

ischemia are compared with the respective data of controls. Obviously the most pronounced change was found in the adenosine fraction of the perfusate, the RSA value of which decreased from a control value of 55×10^{-3} to 3×10^{-3}. The RSA values of inosine and hypoxanthine in the perfusate were only reduced to about half of the control. Furthermore, in the ischemic myocardium the specific activities of cAMP and AMP increased remarkably, whereas those of ATP and ADP were not significantly different.

DISCUSSION

Several lines of investigation suggest that myocardial adenine nucleotides must be considered to be both structurally and functionally compartmentalized [8, 13, 23, 24], although direct experimental evidence has not been obtained as yet. To our knowledge it has not been studied, to what extent adenine nucleotide degradatives such as adenosine, inosine and hypoxanthine, originate from different nucleotide compartments of the heart. One possible means of investigating this problem is suggested by the fact, that in a homogenous biochemical system the specific activities of a precursor and of its immediate metabolic product must be identical [25]. Consequently any difference observed in specific activities under steady state conditions can be taken to indicate compartmentation of the precursor substance.

Based on these considerations, specific activities of adenine nucleotides and their degradatives (adenosine, inosine and hypoxanthine) were determined in isolated perfused hearts and cardiac perfusates after

prelabeling with ^{14}C-adenine and ^{14}C-adenosine. The isolated heart preparation used in our previous studies [3] appears well suited to these investigations since it proved to be metabolically and functionally stable during the experimental period: Tissue concentrations of adenine nucleotides do not decrease measurably, the loss of purines into the perfusate is small and coronary flow as well as myocardial oxygen consumption remain constant.

The most intriguing finding concerns the fact that under normoxic conditions the specific activities of adenosine and cAMP in the perfusate exceeded by far the respective values of the myocardial precursor nucleotides. These differences were observed after prelabeling with both adenine and adenosine. Furthermore, the same pattern of specific activities was obtained when nucleotides were prelabeled with ^{14}C-adenine in presence of different adenine concentrations. It thus appears unlikely that diffusion gradients across the vascular wall during the prelabeling period are responsible for the differences in the specific activities. Our data therefore indicate that adenosine and cAMP released from the well-oxygenated heart are derived from an adenine nucleotide compartment of high specific activity. The low specific activity values of ATP, ADP and AMP determined in these hearts can only represent mean specific activities resulting from the mixing of at least two different nucleotide fractions during tissue extraction.

The existence of differently labeled nucleotide fractions in the myocardium is further supported by two observations: 1. Severe ischemia, which caused – as could be expected [2, 4, 5, 9] – a marked degradation of total adenine nucleotides, led to a pronounced drop in the specific activity of adenosine in the perfusate. It thus appears that the highly labeled adenosine must have been diluted during ischemia with adenosine derived from adenine nucleotides of low specific activity. 2. In the perfusate of normoxic hearts the specific activities of inosine and hypoxanthine were always much lower than the respective values for adenosine. Consequently, a nucleotide fraction of low specific activity serves as precursor for the formation of inosine and hypoxanthine. Furthermore, these data do not support the suggestion [18] that under normoxic conditions inosine is mainly formed from extracellular adenosine.

In view of the similar specific activities of adenosine and cAMP the question arises as to whether adenosine is generated from a highly labeled ATP fraction on a pathway ATP→ADP→AMP→adenosine or/and ATP→cAMP→AMP→adenosine. Our results do not permit to define which of the two pathways is preferentially involved in the formation of adenosine. In this respect, however, it should be noted that the enzymes catalyzing the reactions via cAMP (adenylcyclase, low K_m-phosphodiesterase and 5′-nucleotidase) have been shown to be associated with the particulate or membranous fraction of the heart [1, 17, 20].

Our data do not provide direct information as to the localisation and the size of the highly labeled precursor pool. In view of the distribution of the enzymes catalyzing the formation of adenosine it is reasonable to assume that this pool is localized in the vicinity of the sarcolemma membrane. A preferential localisation of the highly labeled pool in the vascular tissue (e.g. pericytes, endothelial and smooth muscle cells) seems unlikely, since this would imply that adenine phosphoribosyl transferase and adenosine kinase – the enzymes responsible for the incorporation of adenine and adenosine, respectively – are present almost exclusively in the vasculature. The size of the highly labeled ATP pool can be only rather small, because the specific activities of perfusate adenosine and cAMP are about one order of magnitude higher than the specific activity of the adenine nucleotides present in the tissue extracts.

An interesting finding concerns the order of specific activities among the adenine nucleotides. With ^{14}C-adenine as prelabeling substance we found the order to be ATP > ADP > AMP, whereas with ^{14}C-adenosine the specific activities of the nucleotides proved to be almost identical. The reason, why isotope equilibrium was reached only in the case of prelabeling with adenosine, remains unclear at present.

Adenosine has been postulated to exist as such only in the extracellular space [17], a concept which was challenged by Kübler et al. [12]. Our data, too, are difficult to reconcile with this hypothesis. If all the adenosine in the myocardium (2.1 nmoles/g) is localized extracellularly (extracellular space of the isolated heart 0.4 ml/g [15]) the effective concentration of adenosine would be 5.3 μM. This adenosine concentration, however, is known to elicit maximal coronary dilation [3]. Our assumption that only a small fraction of myocardial adenosine can be present in the extracellular space gains additional support by the observation that in the well-oxygenated heart the specific activity of adenosine in the perfusate was more than 5 times higher than the specific activity of adenosine in the myocardium. Hence, when evaluating the role of adenosine in the regulation of coronary flow [2] one has to take into account that extrapolation from tissue levels of adenosine must tend to overestimate the adenosine concentration to which the coronaries are actually exposed.

Acknowledgement. The skillful technical assistance of Mrs. Christa Schäfer-Grunitz is gratefully acknowledged.

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Received July 9, 1976