Pflügers Arch. 369, 1–6 (1977)

Pflügers Archiv
European Journal of Physiology

Release of Adenosine, Inosine and Hypoxanthine from the Isolated Guinea Pig Heart during Hypoxia, Flow-Autoregulation and Reactive Hyperemia*

JÜRGEN SCHRADER, FRANCIS J. HADDY**, and ECKEHART GERLACH

Physiologisches Institut der Universität München, Pettenkoferstrasse 12, D-8000 München 2, Federal Republic of Germany

Summary. In an attempt to test the hypothesis whether adenosine is involved in the regulation of coronary flow, adenosine, inosine and hypoxanthine were measured in the effluent perfusate and in the tissue of isolated guinea pig hearts under various experimental conditions. In addition, the release of ^{14}C-adenosine, ^{14}C-inosine and ^{14}C-hypoxanthine was determined after prelabeling cardiac adenine nucleotides with ^{14}C-adenine.

The decrease in coronary resistance induced by hypoxic perfusion (30% and 20% in the gas phase) and during autoregulation was associated with a considerable increase in the release of adenosine, inosine and hypoxanthine. Under both conditions the concentrations of adenosine in the effluent perfusate were clearly within the coronary vasodilating range of exogenously administered adenosine. The tissue content of adenosine also increased significantly when the perfusion pressure was reduced. The release of ^{14}C-adenosine closely paralleled the changes in coronary resistance during hypoxic perfusion, autoregulation and during reactive hyperemia. The specific activity of adenosine in the effluent perfusate, however, decreased substantially upon reduction of the oxygen supply to the heart, indicating that the release of ^{14}C-adenosine does not provide an absolute measure of total adenosine release by the heart.

Our data indicate that the greater part of the adaptive changes of vascular resistance during hypoxia and autoregulation can be attributed to adenosine which is formed at an enhanced rate under these conditions. However, other factors might be involved as well.

Key words: ^{14}C-adenine – Coronary flow – Adenosine – Inosine – Hypoxanthine – Hypoxia – Autoregulation – Reactive hyperemia.

INTRODUCTION

Among the various factors which are considered to participate in the metabolic regulation of coronary flow [7,8], adenosine has been postulated to play an important role in the adjustment of coronary flow to the oxygen requirement of the myocardium [1,4]. The adenosine hypothesis is supported by recent findings which indicate that adenosine is released even by the well-oxygenated heart [14,16,18] and that its formation is enhanced in association with the vascular dilation following anoxia [10], hypoxia [1,2,4,17] and transient myocardial ischemia [12,15]. The possibility that adenosine could also be involved in the changes of coronary resistance during flow autoregulation has not been investigated to our knowledge, although several explanations concerning the mechanism of autoregulation have been proposed [16,9,11].

Spectrophotometric analysis of adenosine in the coronary effluent usually requires collection and analysis of rather large volumes of coronary venous blood or perfusate [14,15,18]. On the other hand, relatively small samples of coronary effluent are required in order to determine radioactively labeled adenosine which is released after prelabeling myocardial adenine nucleotides with labeled adenine and adenosine [18]. Making use of both procedures, release rates of adenosine and of ^{14}C-adenosine into the cardiac perfusate were determined during hypoxia, autoregulation and reactive hyperemia. The observed changes in the perfusate concentrations of adenosine were related to the alterations in coronary resistance.

* A preliminary report of these studies was given at the VI. Annual Meeting of the "International Study Group for Research in Cardiac Metabolism", Freiburg i. Br., September 1973 and appeared in "Recent advances in studies on cardiac structure and metabolism", Vol. 7, Editors: P. Harris, R. J. Bing, and A. Fleckenstein, pp. 171–175. Urban & Schwarzenberg 1976

** Present address: Uniformed Services University of the Health Sciences, 6917 Arlington Road, Bethesda, Maryland, 20014 U.S.A.

Furthermore it was investigated whether determination of ^{14}C-adenosine yields a suitable and more sensitive measure for the total amount of adenosine released from the heart.

METHODS

Isolated guinea pig hearts were perfused according to the Langendorff technique with a modified Krebs-Henseleit solution as described in previous communications [3,18]. Coronary flow was continuously monitored in each experiment with an electromagnetic flowmeter (Statham, M-4001); the flow probe (IVM, type K-2B) was incorporated into the aortic cannula. Each heart was allowed to equilibrate for 30 min; during this period coronary flow attained stable values of approximately 5 ml/min/g. Hearts which appeared to be in a steady state of cardiac performance – as judged by the heart rate and coronary flow – were then perfused with a medium containing (8-^{14}C)-adenine (62 mCi/mmole) at a concentration of 0.42 µM. Following a labeling period of 35 min, perfusion was continued with an adenine free medium and cardiac perfusate was collected before and after imposing various perturbations on the heart. If not otherwise indicated, perfusate was collected for 5 min, and only when coronary flow was stable.

The oxygen content in the coronary inflow (aortic cannula) and outflow (pulmonary artery) was measured with a Lex O_2-Con (Lexington Instrument Corp.) and myocardial oxygen consumption was calculated from flow and AV O_2-difference.

The analytical procedures and the materials used have already been described in detail [18]. In brief, for radioactivity measurements, nucleosides and purine bases released into the cardiac perfusate were adsorbed onto activated charcoal after carrier amounts (0.05 µmoles) of adenosine, inosine and hypoxanthine had been added. After elution from the charcoal with a mixture of pyridine and ethanol, the purine compounds were separated by thin layer chromatography (Sil-G/UV_{254}). The UV-absorbing bands representing adenosine, inosine and hypoxanthine were then scraped off the plates and assayed for radioactivity. Measurements of the release of radioactively labeled purines from the hearts were expressed as cpm/min/g tissue and will be referred to as "radiometric analysis".

The relative specific activity values of adenosine, inosine and hypoxanthine in the coronary perfusate were calculated by relating the specific activities of each purine to the specific activity of the precursor adenine in the perfusion medium [18].

In separate experiments absolute concentrations of adenosine, inosine and hypoxanthine in the heart and the amounts of these compounds released into the coronary effluent were determined spectrophotometrically [18]. Values for adenosine and its degradative products in the perfusate are expressed either as release rate (nmoles/min/g) or concentration (nmoles/l). Reported data are not corrected for losses (about 20%) incurred during the different steps of analysis.

RESULTS

Sensitivity of the Coronary Arteries to Adenosine. Figure 1 shows the dose-response relationship for adenosine as determined in the isolated non-working guinea pig heart. Dilation of the coronaries started at an adenosine concentration of about 5×10^{-9} M and reached a maximum at a concentration of

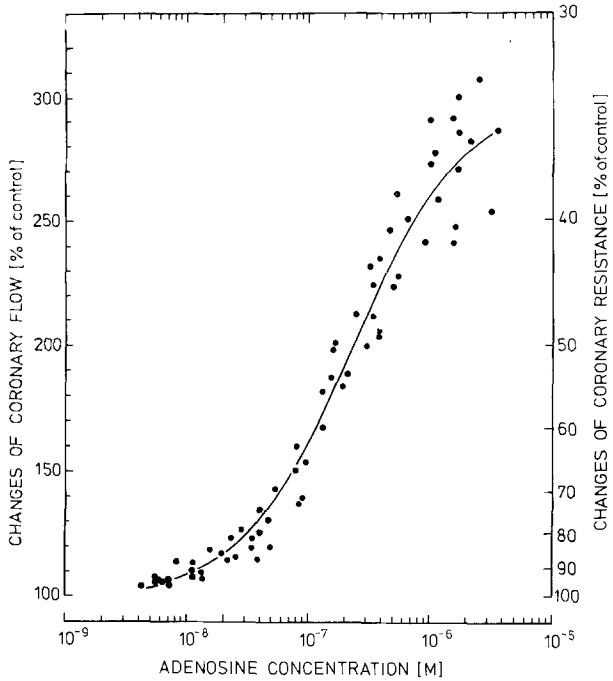

Fig. 1. Dose response relation for changes of coronary flow or coronary resistance caused by adenosine. Perfusion pressure was maintained at 60 cm H_2O when changes in flow were monitored. Coronary flow was kept constant, when perfusion pressure was monitored. During perfusion with constant volume, the initial flow was adjusted to yield a perfusion pressure of 60 cm H_2O. Resistance was then calculated

$2-5 \times 10^{-6}$ M. The hearts exhibited the same responsiveness to adenosine when perfusion was carried out at constant pressure or at constant flow.

Hypoxia. The influence of arterial hypoxia on coronary flow and release of adenosine, inosine and hypoxanthine was studied by lowering the oxygen content in the perfusion medium (equilibration with 30% and 20% in the gas phase). As shown in Table 1, hypoxia was associated with a substantial increase in the release of adenosine and its degradatives. The perfusate concentration of adenosine, inosine and hypoxanthine also increased considerably.

The release of ^{14}C-adenosine and its labeled degradatives was also augmented by hypoxia, after cardiac adenine nucleotides had been prelabeled with ^{14}C-adenine (Fig. 2). The hypoxia induced increase in the release of labeled purines was accompanied by a progressive rise in coronary flow. A reduction in the oxygen content to 20% led to a 3.5-fold increase in the liberation of ^{14}C-adenosine. In contrast, adenosine measured spectrophotometrically (Table 1) increased 33-fold. Consequently, the specific activity of adenosine in the perfusate continuously decreased when the oxygen supply to the heart was reduced

Table 1. Effects of hypoxic perfusion and alterations in perfusion pressure on release rates (nmoles/min/g) and perfusate concentrations (nmoles/l) of adenosine, inosine and hypoxanthine. Perfusion pressure was maintained at 60 cm H_2O during variations of oxygen content. Perfusate was equilibrated with 95% O_2 – 5% CO_2 during variations in perfusion pressure. Mean values ± S.E.M. ($n = 5$)

		Adenosine		Inosine		Hypoxanthine	
		(nmoles/min/g)	(nmoles/l)	(nmoles/min/g)	(nmoles/l)	(nmoles/min/g)	(nmoles/l)
% O_2 in gas phase	95%	0.048 ± 0.002	4.0 ± 0.3	0.37 ± 0.1	59 ± 5.7	0.057 ± 0.02	9.5 ± 5.0
	30%	0.474 ± 0.011	33.8 ± 5.9	3.65 ± 1.6	258 ± 98.0	0.220 ± 0.06	15.3 ± 2.9
	20%	1.595 ± 0.170	88.3 ± 7.3	6.00 ± 1.4	333 ± 72.0	0.490 ± 0.16	27.3 ± 8.3
Perfusion pressure (cm H_2O)	80	0.056 ± 0.008	3.9 ± 0.8	0.33 ± 0.1	45 ± 6.7	0.049 ± 0.02	6.5 ± 1.6
	60	0.048 ± 0.002	4.0 ± 0.3	0.37 ± 0.1	59 ± 5.8	0.057 ± 0.02	9.5 ± 5.0
	40	0.076 ± 0.010	15.0 ± 0.9	0.33 ± 0.04	65 ± 6.1	0.032 ± 0.01	6.4 ± 1.9
	20	0.256 ± 0.038	78.3 ± 0.9	1.43 ± 0.3	446 ± 95.0	0.200 ± 0.05	61.0 ± 13.3

Table 2. Effects of alterations in perfusion pressure and oxygen content of the perfusion medium on the relative specific activities (RSA $\times 10^{-3}$) of adenosine, inosine and hypoxanthine in the coronary perfusate. (For calculation see Methods). Mean values ± S.D.; $n = 3$

	20 cm H_2O 95% O_2	40 cm H_2O 95% O_2	60 cm H_2O 95% O_2	60 cm H_2O 30% O_2	60 cm H_2O 20% O_2
Adenosine	17.6 ± 2.6	45.1 ± 4.2	54.6 ± 3.1	11.9 ± 2.1	6.1 ± 0.5
Inosine	6.6 ± 1.4	4.7 ± 0.5	3.3 ± 0.3	3.1 ± 0.8	2.6 ± 1.2
Hypoxanthine	4.6 ± 0.9	3.7 ± 0.8	4.1 ± 1.2	3.6 ± 0.3	2.5 ± 0.9

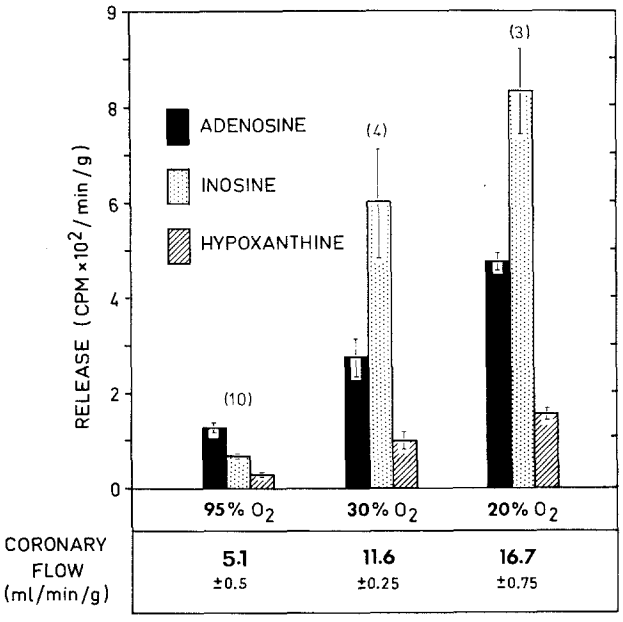

Fig. 2. Changes in coronary flow and rate of release of ^{14}C-adenosine, ^{14}C-inosine and ^{14}C-hypoxanthine during normoxia and graded hypoxia. Oxygen content of the perfusate was altered by equilibrating the perfusate with a gas mixture containing 95, 30 or 20% O_2, 5% CO_2, balance being N_2. Vertical bars represent ± S.E.M.

(Table 2). On the other hand, the specific activity of inosine and hypoxanthine remained almost unchanged in this situation.

Autoregulation. The isolated guinea pig heart maintained a rather constant coronary flow upon reduction of perfusion pressure particularly in the range of 60 to 20 cm H_2O (Fig. 3). This was the result of active coronary vasodilation. Upon decreasing the perfusion pressure the oxygen supply to the hearts became reduced and this condition was associated with a fall in myocardial oxygen consumption (Fig. 3).

As shown in Table 1, a decrease in perfusion pressure from 60 to 20 cm H_2O resulted in a progressive increase in the rate of adenosine release and in the perfusate concentration of adenosine. Rates of release of inosine and hypoxanthine as well as their concentrations also increased during this experimental condition. Elevation of the perfusion pressure from 60 to 80 cm H_2O, did not cause a significant change in the liberation of the different purine compounds. Measurements of the myocardial tissue levels of adenosine, inosine and hypoxanthine revealed that a reduction in perfusion pressure to 20 cm H_2O was associated

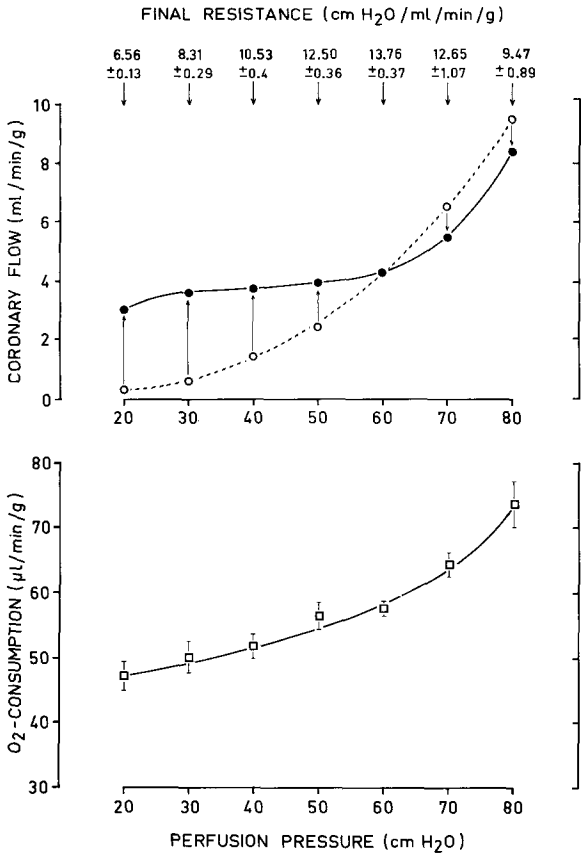

Fig. 3. *Upper panel:* Pressure flow relationship in isolated perfused guinea pig hearts. Pressure changes were initiated from a control perfusion pressure of 60 cm H_2O. Open circles represent flow changes immediately following abrupt pressure changes. Closed circles represent stable flow values reached 1 to 3 min after pressure changes. Final resistance ± S.E.M. (n = 10) was calculated from stable flow values at each pressure level. *Lower Panel:* Myocardial oxygen consumption at different perfusion pressures (n = 8; ± S.E.M.)

Table 3. Content of adenosine, inosine and hypoxanthine in isolated hearts at two different perfusion pressures. Mean values ± S.E.M.

Perfusion pressure (cm H_2O)	Adenosine (nmoles/g)	Inosine (nmoles/g)	Hypoxanthine (nmoles/g)
60	2.13 ± 0.14 (n = 30)	0.98 ± 0.07 (n = 28)	1.26 ± 0.13 (n = 27)
20	3.12 ± 0.25 (n = 5)	5.89 ± 1.31 (n = 5)	3.47 ± 0.93 (n = 5)

with a significant increase in the tissue content of adenosine and its degradatives (Table 3).

Upon reduction of perfusion pressure the release of ^{14}C-adenosine, ^{14}C-inosine and ^{14}C-hypoxanthine into the cardiac perfusate was also enhanced (Fig. 4). It should be noted, however, that ^{14}C-inosine increased more steeply than ^{14}C-adenosine and ^{14}C-hypoxanthine when the perfusion pressure was reduced below 40 cm H_2O. As in experiments with hypoxic perfusion, the specific activity of adenosine in the perfusate decreased when the perfusion pressure was reduced (Table 2). In contrast, the specific activity of inosine appeared to increase slightly, whereas the values for hypoxanthine remained unchanged.

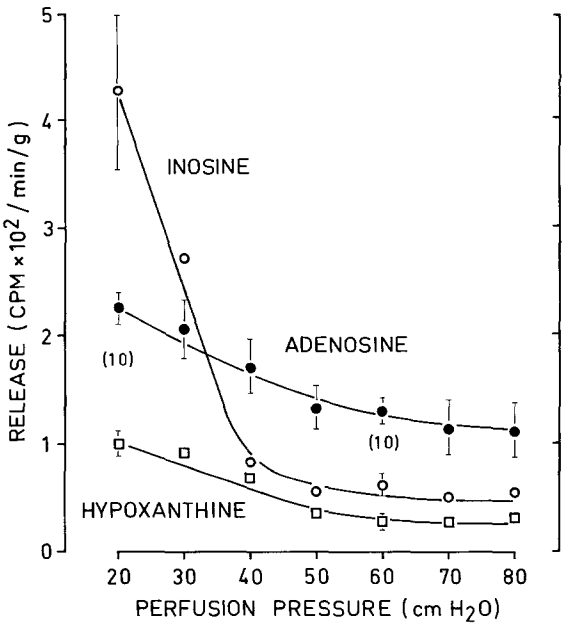

Fig. 4. Release of ^{14}C-adenosine, ^{14}C-inosine and ^{14}C-hypoxanthine from the myocardium as a function of perfusion pressure. In individual experiments perfusion pressure was either successively raised from 20 to 60 cm H_2O (n = 2) or lowered from 80 to 20 cm H_2O (n = 3) or changed abruptly from 60 to 20 cm H_2O (n = 5). At each perfusion pressure, effluent was collected (5 min) for analysis after flow became stable. Vertical bars represent ± S.E.M.

Reactive Hyperemia. Spectrophotometric measurements of adenosine and its degradatives in the perfusate collected during reactive hyperemia following short periods of coronary occlusion were not possible, because the amounts of adenosine released were below detection limits of the method. Therefore, only the release of ^{14}C-adenosine, ^{14}C-inosine and ^{14}C-hypoxanthine into the coronary perfusate could be determined. It is evident from Figure 5 that the flow responses were paralleled by increased release rates of ^{14}C-adenosine, as well as of ^{14}C-inosine and of ^{14}C-hypoxanthine. It is also apparent that in all cases preocclusion values for the release of labeled purine compounds were reached when coronary flow returned to control values.

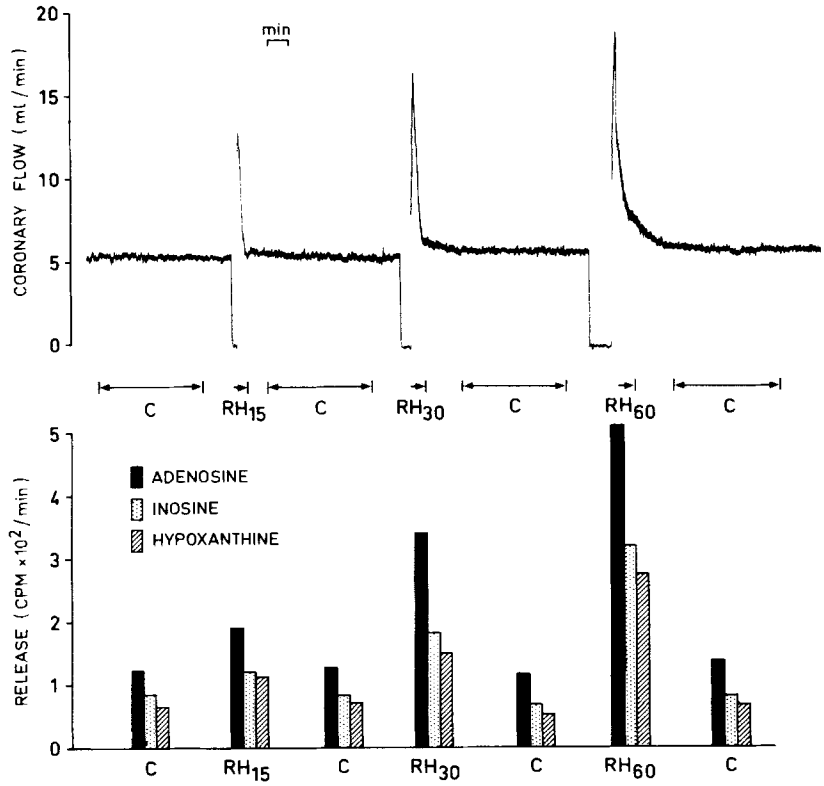

Fig. 5
Release of labeled ^{14}C-adenosine, ^{14}C-inosine and ^{14}C-hypoxanthine into the coronary effluent during reactive hyperemia (*RH*) following coronary occlusion for 15, 30 and 60 s. Purine compounds were analyzed in the effluent during the period of reactive hyperemia as well as during the control period (*C*) before and after each flow response, as indicated by the arrows

DISCUSSION

The intrinsic ability of the heart to regulate coronary flow appears to be closely related to the supply and demand of the tissue for oxygen [5]. Any condition which leads to an imbalance between myocardial oxygen supply and demand is postulated to alter the formation and release of adenosine which in turn causes an adjustment of coronary flow [1,15]. The experimental conditions chosen in the present study to reduce oxygen supply to the heart (hypoxia, low perfusion pressure) were found to be associated with an enhanced release of adenosine into the coronary perfusate. In addition, occlusion of coronary inflow for short periods was followed by an augmented release of ^{14}C-adenosine from prelabeled hearts during the period of reactive hyperemia.

A quantitative evaluation of the role of adenosine in coronary flow regulation would require measurements of the adenosine concentration in the vicinity of the coronary resistance vessels. At present, however, only determinations of tissue content, coronary and pericardial perfusate concentrations of adenosine are feasible. Concerning measurements of the myocardial content of adenosine, we have recently suggested that not all the adenosine can be present within the extracellular space [18]. Therefore, extrapolation from tissue levels to the adenosine concentration in the extracellular space tends to overestimate the amount of this nucleoside to which the coronaries are exposed [18,19]. On the other hand, measurements of the adenosine concentration in the coronary effluent perfusate underestimate the quantity of adenosine present at the site of the smooth muscle cell, for the following reasons: i) Adenosine is constantly washed out into the vascular space and thus becomes diluted. ii) A fraction of adenosine is taken up again by the myocardium and another fraction is deaminated to inosine [20]. It thus appears that these factors may limit conclusions concerning the validity of the adenosine hypothesis when quantitative considerations are based on measurements of tissue content or perfusate concentrations of adenosine.

Regardless of these limitations our data show that during autoregulation and hypoxic perfusion the perfusate concentrations of adenosine increase considerably and are well within the dilatory range of exogenously applied adenosine. Even in normoxic hearts, the perfusate concentration of adenosine (4×10^{-9} M) is approximately the same as the threshold concentration for coronary vasodilation. In addition, the tissue content of adenosine increases during autoregulation and an increase in myocardial adenosine content has been observed by others during hypoxic perfusion [17]. Thus, these findings support the hypothesis that adenosine can be involved in the

adjustment of coronary flow during hypoxia as well as during autoregulation. However, other factors may also play a role [7, 8]. Consistant with this idea is our observation, that an increase in perfusion pressure from 60 to 80 cm H_2O was associated with an autoregulatory response, although there were no significant changes in the adenosine concentration of the perfusate.

Release of ^{14}C-adenosine and its labeled degradatives was also found to be augmented during hypoxia, autoregulation and reactive hyperemia. The changes observed during the latter condition were detected in perfusate samples collected for as little as 30 s, and proved to be proportional to the time of coronary occlusion. Measurements of the specific activity of adenosine in the perfusate, however, revealed that during hypoxia and autoregulation the specific activity of adenosine decreases considerably. Thus, despite its sensitivity and simplicity, radiometric analysis of adenosine cannot provide a quantitative estimate of the amount of adenosine released by the heart. The prelabeling technique with ^{14}C-adenine, however, may be valuable when attempting to measure changes in the release of adenosine during rapid and transient adaptive vascular adjustments.

We previously suggested [18] that adenosine released into the perfusate is most probably derived from at least two adenine nucleotide fractions of the heart. Our present findings are in accord with this view: During autoregulation and hypoxia, the specific activity of adenosine in the perfusate decreased considerably, indicating that a cardiac adenine nucleotide fraction of low specific activity contributes to the formation of adenosine under these conditions.

Acknowledgement. The skilful technical assistance of Mrs. Christa Schäfer-Grunitz and Mr. Horst Malotta is gratefully acknowledged.

REFERENCES

1. Berne, R. M.: Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. Amer. J. Physiol. **204**, 317–322 (1963)
2. Berne, R. M., Rubio, R., Duling, B. R., Wiedmeier, V. T.: Effects of acute and chronic hypoxia on coronary blood flow. Advanc. Cardiol **5**, 56–66 (1970)
3. Bünger, R., Haddy, F. J., Querengässer, A., Gerlach, E.: An isolated guinea pig heart preparation with in vivo like features. Pflügers Arch. **353**, 317–326 (1975)
4. Gerlach, E., Deuticke, B., Dreisbach, R. H.: Der Nucleotid-Abbau im Herzmuskel bei Sauerstoffmangel und seine mögliche Bedeutung für die Coronardurchblutung. Naturwissenschaften **50**, 228–229 (1963)
5. Granger, H. J., Shepherd, A. P.: Intrinsic microvascular control of tissue oxygen delivery. Microvasc. Res. **5**, 49–72 (1973)
6. Guyton, A. C., Ross, J. M., Carrier, O., Walker, J. R.: Evidence for tissue oxygen demand as a major factor causing autoregulation. Circulat. Res. (Suppl. I) **14**, 60–80 (1964)
7. Haddy, F. J., Scott, J. B.: Metabolically linked chemicals in local regulation of blood flow. Physiol. Rev. **48**, 688–707 (1968)
8. Haddy, F. J., Scott, J. B.: Metabolic factors in peripheral circulatory regulation. Fed. Proc. **34**, 2004–2011 (1975)
9. Johnson, P. C.: Review of previous studies and current theories of autoregulation. Circulat. Res. (Suppl. I) **14**, 1–9 (1964)
10. Katory, M., Berne, R. M.: Release of adenosine from anoxic hearts. Circulat. Res. **19**, 420–425 (1966)
11. Mosher, P., Ross, J., McFate, P. A., Shaw, R. F.: Control of coronary blood flow by an autoregulatory mechanism. Circulat. Res. **14**, 250–259 (1964)
12. Olsson, R. A.: Changes in content of purine nucleosides in canine myocardium during coronary occlusion. Circulat. Res. **26**, 301–306 (1970)
13. Olsson, R. A.: Myocardial reactive hyperemia. Circulat. Res. **37**, 263–270 (1975)
14. Rubio, R., Berne, R. M.: Release of adenosine by the normal myocardium in dogs and its relation to the regulation of coronary resistance. Circulat. Res. **25**, 407–415 (1969)
15. Rubio, R., Berne, R. M., Katori, M.: Release of adenosine in reactive hyperemia of the dog heart. Amer. J. Physiol. **216**, 56–62 (1969)
16. Rubio, R., Wiedmeier, V. T., Berne, R. M.: Nucleoside-phosphorylase: localisation and role in the myocardial distribution of purines. Amer. J. Physiol. **222**, 550–555 (1972)
17. Rubio, R., Wiedmeier, V. T., Berne, R. M.: Relationship between coronary flow and adenosine production and release. J. Molec. Cell. Cardiol. **6**, 561–566 (1974)
18. Schrader, J., Gerlach, E.: Compartmentation of cardiac adenine nucleotides and formation of adenosine. Pflügers Arch. **367**, 129–135 (1976)
19. Snow, J. A., Olsson, R. A., Gentry, M. K.: Myocardial: blood purine nucleoside concentration ratios in canine myocardium. In: Current topics in coronary research (C. M. Bloor and R. A. Olsson, eds.). New York-London: Plenum Press 1973
20. Wiedmeier, V. T., Rubio, R., Berne, R. M.: Incorporation and turnover of adenosine-U-^{14}C in perfused guinea pig myocardium. Amer. J. Physiol. **223**, 51–54 (1972)

Received November 5, 1976