PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 176, 22–26 (1984)

Effect of Perfusate pH on Coronary Flow and Adenosine Release in Isolated Rabbit Heart[1,2] (41836)

S. JAMAL MUSTAFA[3] AND M. MAHY MANSOUR[4]

Department of Pharmacology, School of Medicine, East Carolina University, Greenville, North Carolina 27834

Abstract. This study was undertaken in an attempt to further understand the relationship between adenosine and H^+ ion. Using Langendorff hearts from male rabbits, the perfusion fluid pH was lowered from 7.4 to 7.1 and 6.8 with CO_2. A 31 and 86% increase in coronary flow with a simultaneous increase in the release of adenosine by 61 and 128% was observed at pH 7.1 and 6.8, respectively. A direct relationship between adenosine release and coronary flow with a correlation coefficient of 0.99 was found at pH values of 7.4, 7.1, and 6.8. The degradation products of adenosine namely inosine and hypoxanthine were unchanged at 7.1 and 6.8 from 7.4. These data support a role for adenosine in the regulation of coronary flow and suggest a relationship between adenosine and H^+ ion.

Adenosine, a vasoactive metabolite plays an important role in the physiological regulation of coronary blood flow (1). Several investigators (2–4) support the view that cations such as hydrogen ion and potassium ion may influence the adenosine-induced increases in coronary flow. Additionally, adenosine antagonists (theophylline and aminophylline) have been reported (5–11) to partially attenuate the reactive hyperemia which occurs subsequent to the release of coronary inflow occlusion. However, similar concentrations of these adenosine antagonists have been reported (5) to abolish the increase in flow responses to exogenous adenosine. Further, a decrease in pH (12, 13) along with the release of adenosine (9–11, 14) have been observed during myocardial ischemia. These investigations suggest that the response to adenosine may be modified by other factors during ischemic insult and perhaps this is the reason why the concentration of adenosine antagonists which effectively inhibit the response to exogenous adenosine are unable to block reactive hyperemia completely.

Recently, we have reported (15) that the lowering of perfusion fluid pH from 7.4 to 6.8 can significantly inhibit the uptake of adenosine. Thus, this investigation was an attempt to test the hypothesis that lowering of the perfusion pH can inhibit the myocardial uptake of adenosine leading to an increased release of adenosine in the perfusate. Therefore, we measured the release of adenosine by altering the pH of the perfusion solution from 7.4 to 7.1 and 6.8 with a simultaneous measurement of coronary flow from rabbit heart using the Langendorff preparation.

Methods. Hearts from rabbits, 1–1.5 kg, of local strain were quickly excised and perfused through the cannulation of the aorta using a Krebs–Ringer bicarbonate buffer containing (in m*M*): glucose, 5.5; pyruvate, 2.0; NaCl, 127.5; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; and $NaHCO_3$, 24.9. The perfusion fluid was maintained at 37°C and equilibrated with 95% O_2 + 5% CO_2 (pH 7.4). The perfusion pressure was maintained at a height of 65 cm of H_2O throughout the experiment. Changes in the pH of the perfusion solution were maintained by changing the proportions of O_2 and CO_2 to give the desired pH. The concentration of CO_2 required (<10%) to change the pH to 7.1 and 6.8 was not enough to induce hypoxia and therefore change the coronary flow and adenosine release.

The aortic inflow rate, which equals to the total coronary flow was monitored with an electromagnetic flow meter (Biotronex model BL-610), the flow probe was interposed in the perfusion line several centimeters above the heart. The flow transducer was connected to

[1] Supported by HL 27339.

[2] This work was conducted at the University of South Alabama Medical School, Mobile, Ala.

[3] To whom reprint requests should be addressed.

[4] Present address: Department of Physiology, Faculty of Medicine, Al-Azhar, University, Cairo, Egypt.

0037-9727/84 $1.50

a polygraph (Beckman model R411) for a continuous recording of the total mean coronary flow. The apex of the heart was connected by means of a thread to a force displacement transducer (Grass FT .03) and connected to a physiograph for continuous recording of the rate and force of contraction.

An initial 30-min period was allowed for the stabilization of the heart before actual experimentation. The control response for flow, heart rate, and contractions were recorded. A period of 10 min was allowed after each change in the pH of the perfusion fluid to achieve maximal effect before collecting the subsequent 10-min samples of the perfusate from the heart. Changes in coronary flow, heart rate, and force of contraction were recorded. The preparation was checked for its autoregulatory response. A 30-sec occlusion of inflow resulted in more than twofold increase in coronary flow at the release of occlusion. The samples were analyzed for adenosine, inosine, and hypoxanthine according to the method described by Watkinson *et al.* (16) using enzymatic analysis and recording the absorbance changes on dual-wave length, double-beam spectrophotometer (Aminco model DW-2). Student's *t* test was used for the level of significance and the results are expressed as the mean ± SE.

Results and Discussion. The results show an increased release of adenosine when the hydrogen ion concentration is raised in perfusion medium. Figure 1 shows the release of adenosine, inosine, and hypoxanthine at pH values of 7.4, 7.1, and 6.8. The amount of adenosine released into the perfusate increased from a control value of 1.68 ± 0.17 nmole/g wet weight/10 min of collected sample at a pH of 7.4 to 2.7 ± 0.29 nmole/g wet weight/10 min at pH 7.1, a 60% rise. This value was raised to 128% (3.83 ± 0.36 nmole/g wet weight/10 min) from its control value by changing the pH to 6.8 using CO_2. On the other hand the concentrations of inosine and hypoxanthine in the coronary perfusates were unchanged. Further, the sum of adenosine, inosine, and hypoxanthine was unchanged at pH 7.1 but slightly higher (insignificant) at pH 6.8 from pH 7.4. It has been reported (1) that the dephosphorylation of adenosine-5′-monophosphate is the major pathway for the production of adenosine, therefore, the expected changes will take place in the intracellular adenine nucleotide pool if the activities of nucleoside phosphorylase and xanthine oxidase are unaltered at various pH values and not in the sum of perfusate adenosine, inosine, and hypoxanthine. Our data support this view. The values reported here for adenosine and its degradative products are similar to those reported by Fredholm *et al.* (17) using isolated rabbit hearts. The concentration of adenosine at pH 6.8 was significantly higher than the concentration at pH 7.1 and 7.4.

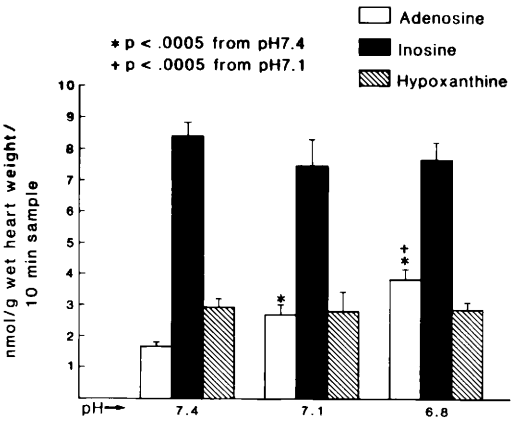

FIG. 1. Release of adenosine, ionsine, and hypoxanthine in the perfusates of isolated rabbit hearts at pH values of 7.4, 7.1, and 6.8. The values are represented as means ± SE from 10–17 different experiments.

Figure 2 shows the relationship between adenosine release, coronary flow, and pH changes. Using adenosine release (nmole/g wet heart weight/10 min sample) or coronary flow (ml/min/g wet heart weight) as *y* axis, and pH changes as *x* axis, a positive correlation ($r = 0.99$) was found at pH values of 7.4, 7.1, and 6.8. Also, a positive correlation ($r = 0.99$) was found between adenosine release (*x* axis) and coronary flow (*y* axis) at all the pH values (not shown). This is similar to the observation of several investigators (1, 16, 18, 19) where a direct relationship between adenosine release, coronary flow, and oxygen consumption has been reported. In our experiments, the heart rate was not altered during the pH changes. The force of myocardial contraction was not affected by lowering the pH to 7.1 but was significantly lower at pH 6.8. It has been reported (20, 21) that changes in myocardial contraction can affect the coronary blood flow. However, in our studies the degree

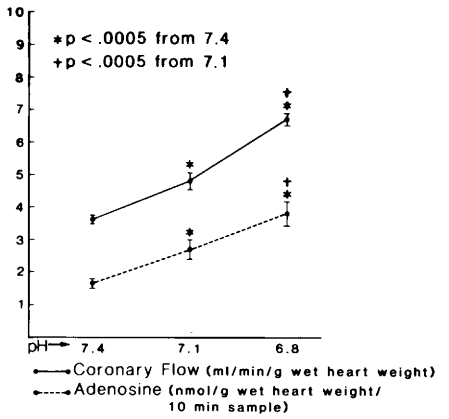

FIG. 2. Correlation between the release of adenosine or coronary flow from the rabbit hearts at pH values of 7.4, 7.1, and 6.8. The values are represented as means ± SE from 10–17 experiments. A correlation coefficient of 0.99 was found for adenosine release or coronary flow at different pH values.

of correlation between adenosine and coronary flow is 98% at all the pH values. It is unlikely that the changes in contractility caused the increase in coronary flow and adenosine release at pH 6.8. In fact, the decrease in myocardial contractility at pH 6.8 would be expected to lower the metabolic activity and therefore, decrease the release of adenosine. Further, the fact that the parallel increase in coronary flow and adenosine release at pH 7.1 was not accompanied by a change in contractility rules out its involvement in the observed increases in coronary flow.

Several investigators (2, 22–24) have studied the effect of H^+ ion concentration on the vascular response to adenosine but this is the first report where the actual release of adenosine has been measured at varying pH values. Degenring (23) using isolated guinea pig hearts studied the effect of acidosis and alkalosis in normoxic and hypoxic hearts with a simultaneous measurement of myocardial adenine nucleotides and nucleosides and coronary flow. He reported (23) a significant increase in coronary flow and myocardial adenosine both in normoxic and hypoxic hearts under acidosis (pH 7.0), whereas the reverse was true for alkalosis (pH 7.8). The pH in this case (23) was adjusted by using HCl or NaOH. Our data are in agreement with the studies of Degenring (23). However, on a quantitative basis, our data show an increase in coronary flow of 31% and an adenosine release of 61% at pH 7.1, whereas Degenring (23) found only an increase of 13% in coronary flow and an increase of 25% in tissue adenosine under normoxic conditions at pH 7.0. This is possibly due to the fact that the acidosis in our study was induced by increasing the CO_2 and in the study by Degenring (23) HCl was used to induce acidosis. It is likely that CO_2 has dual effects, indirectly by increasing the H^+ ion concentration and directly on the coronary vessels (25). However, it is quite possible that CO_2 has a better diffusibility due to the higher lipid solubility. In fact, this laboratory has reported (24) earlier that bovine large and small coronary vessels relaxed more when the pH of the media was adjusted by CO_2 rather than by adjusting the bicarbonate. In some of the experiments, where the acidosis was induced by HCl, the increase in coronary flow was less dramatic than with CO_2 (data not reported).

Degenring (23) in his studies measured the myocardial levels of adenosine during various experimental conditions. In the light of new information (26), more than 90% of adenosine in the tissue is intracellular and therefore, may not reflect the interstitial concentration of adenosine available at the resistance vessel site. In the present study, the amount of adenosine measured in perfusates is an appropriate reflection of interstitial concentration of adenosine available for vasodilation. There is a linear correlation between the amount of adenosine released into the perfusates and coronary flow at pH 7.4, 7.1, and 6.8. Our data, therefore, support a role for adenosine under varying H^+ ion concentrations. Similar results have recently been reported by Downing *et al.* (27), who showed an increased response in hypercapnic acidosis to exogenous adenosine both in normal and diabetic lambs and attributed these responses to changes in H^+ ion concentration.

This study supports our earlier observations (15) where we found an inhibition in the uptake of adenosine in cultured heart cells by increasing the H^+ ion concentration. In the earlier report (15), the pH changes were carried out using an acid or base. In this investigation the pH changes were carried out by altering the perfusate CO_2. This change in H^+ ion concentration would result in the inhibition in

the uptake of adenosine (endogenous), thereby increasing its concentration in the extracellular fluid as reflected in perfusate adenosine concentrations and thus an increase in coronary flow. It is clear from these studies that increase in H^+ ion concentration whether induced by CO_2 or an acid (HCl) can inhibit the uptake of adenosine into the cardiac cells and thereby increase its extracellular concentration to which the resistance vessels are exposed. However, other possibilities such as the effect of increased CO_2 on the metabolism of adenosine in the heart, increased binding of adenosine to its receptor (28) or an interaction between H^+ ion and Ca^{2+} at the level of vascular smooth muscle cannot be ruled out from these studies.

In conclusion, the present study demonstrates that the lowering of the perfusate pH from 7.4 to 7.1 and 6.8 results in an increase in coronary flow with a simultaneous increase in the release of adenosine from the heart. These data provide an explanation as to why theophylline and aminophylline are unable to antagonize the reactive hyperemic response. This is due to the fact that in reactive hyperemia the added influence of acidosis leads to levels of adenosine higher than the blocking capacity of antagonist. However, adenosine antagonists at similar concentrations can inhibit the response to exogenous adenosine due to the absence of acidosis (5–8). The data further support a role for adenosine in the regulation of coronary flow and suggest a relationship between adenosine and H^+ ion. The exact nature of this relationship is not known and needs further investigation.

The authors express their appreciation to Mrs. Linda Pope for typing this manuscript.

1. Berne RM. The role of adenosine in the regulation of coronary blood flow. Circ Res **47**:807–813, 1980.
2. Merrill GF, Haddy FJ, Dabney JM. Adenosine, theophylline, and perfusate pH in the isolated, perfused guinea pig heart. Circ Res **42**:225–229, 1978.
3. Young MA, Merrill GF. Differential effects of adenosine and hypoxia on potassium-induced dilation in the isolated, perfused guinea pig heart. Blood Vessels **19**:292–300, 1982.
4. Merrill GF, Young MA, Tozzi CA, Grosso PC, Marcus KM. Adenosine coronary vasodilation and sympathetic adrenergic vasoconstriction during altered CO_2. Artery **10**:395–411, 1982.
5. Afonso S, Ansfield TJ, Berndt TB. Coronary vasodilatation responses to hypoxia before and after aminophylline. J Physiol (London) **221**:589–599, 1972.
6. Bittar N, Pauly TJ. Myocardial reactive hyperemia responses in the dog after aminophylline and lidoflazine. Amer J Physiol **220**:812–815, 1971.
7. Eiken E, Wilcken DEL. Myocardial reactive hyperemia in conscious dogs: Effect of dipyridamole and aminophylline on responses to four and eight seconds coronary artery occlusion. Aust J Exp Biol Med Sci **51**:617–630, 1973.
8. Eikens E, Wilcken DEL. The effect of dipyridamole and aminophylline on responses to sixty-second coronary artery occlusion in dogs. Aust J Exp Med Sci **51**:631–642, 1973.
9. Olsson RA. Changes in content of purine nucleosides in canine myocardium during coronary occlusion. Circ Res **26**:301–306, 1970.
10. Olsson RA, Snow JA, Gentry MK. Adenosine metabolism in canine myocardial reactive hyperemia. Circ Res **42**:358–362, 1978.
11. Rubio R, Berne RM, Katori M. Release of adenosine in reactive hyperemia of the dog heart. Amer J Physiol **216**:56–62, 1969.
12. Eichenhoff JE, Hafkenshiel JH, Landmesser CM. The coronary circulation in the dog. Amer J Physiol **148**:582–596, 1947.
13. Scott JB, Radawski D. Role of hyperosmolarity in the genesis of active and reactive hyperemia. Circ Res **28**(Suppl 1):26–32, 1971.
14. Raberger B, Schultz W, Kraupp O. Coronary reactive hyperemia and coronary dilator action of adenosine during normal respiration and hypercapnic acidosis in the dog. Clin Exp Pharmacol Physiol **2**:373–382, 1975.
15. Mustafa SJ. Effect of varying pH (H^+) on the metabolism of adenosine in isolated heart cells. J Mol Cell Cardiol **12**:335–346, 1980.
16. Watkinson WP, Foley DH, Rubio R, Berne RM. Myocardial adenosine formation with increased cardiac performance in the dog. Amer J Physiol **236**:H13–H21, 1979.
17. Fredholm BB, Hedquist P, Vernet L. Release of adenosine from the rabbit heart by sympathetic nerve stimulation. Acta Physiol Scand **106**:381–382, 1979.
18. Berne RM, Foley DH, Watkinson WP, Wayne L, Miller H, Winn R, Rubio R. Role of adenosine as a mediator of metabolic vasodilation in the heart and brain: A brief overview and update. In Baer HP, Drummond GI, ed. Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides. New York, Raven Press, pp117–126, 1979.
19. Miller WL, Belardinelli L, Bacchus A, Foley DH, Rubio R, Berne RM. Canine myocardial adenosine and lactate production, oxygen consumption and coronary blood flow during stellate ganglia stimulation. Circ Res **45**:708–718, 1979.

20. Gregg DE. Coronary circulation in health and disease. Philadelphia, Pa., Lea & Febiger, 1950.
21. Sabiston DC Jr, Gregg DE. Effect of cardiac contraction on coronary blood flow. Circulation **15**:14–20, 1957.
22. Molnar JI, Scott JB, Frohlich ED, Haddy FJ. Local effects of various anions and H^+ on dog limb and coronary vascular resistances. Amer J Physiol **203**:125–132, 1962.
23. Degenring FH. The effect of acidosis and alkalosis on coronary flow and cardiac nucleotide metabolism. Basic Res Cardiol **71**:287–290, 1976.
24. Mustafa SJ, Ghai G. Effect of adenosine on the relaxation of coronary arteries at varying pH values. Basic Res Cardiol **76**:380–386, 1981.
25. Turnheim K, Pittner H, Kolassa N, Kraupp O. Relaxation of coronary artery strips by adenosine and acidosis. Eur J Pharmacol **41**:217–220, 1977.
26. Olsson RA, Saito D, Steinhart CR. Compartmentalization of the adenosine pool of dog and rat hearts. Circ Res **50**:617–626, 1982.
27. Downing SE, Lee JC, Weinstein EM. Coronary dilator actions of adenosine and CO_2 in experimental diabetes. Amer J Physiol **243**:H252–H258, 1982.
28. Dutta P, Mustafa SJ. Binding of adenosine to the crude plasma membrane fraction isolated from dog coronary and carotid arteries. J Pharmacol Exp Ther **214**:496–502, 1980.

Received July 5, 1983. P.S.E.B.M. 1984, Vol. 176.
Accepted January 24, 1984.