The Effect of Alpha Adrenergic Block on Adenosine- and ATP-induced Coronary Vasodilation[1] (37626)

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A current theory of regulation of coronary blood flow is that of adenosine-induced coronary vasodilation (1). In particular, the hypothesis that a local increase in the extracellular concentration of adenosine is responsible for the reactive hyperemia of temporary coronary occlusion has gained support from the recent work of several investigators (2, 3). Although there is evidence that adenosine is formed through the degradation of the adenine nucleotides in response to myocardial hypoxia (3), the mechanisms by which coronary vasodilation is produced is unknown. A recently reported study in isolated guinea pig and rat hearts suggested that adenosine causes coronary vasodilation by inhibition of alpha adrenergic activity in the coronary resistance vessels and that the effect can be competitively blocked by alpha adrenergic blockade (4). Since myocardial reactive hyperemia has been shown to be unaffected by alpha adrenergic blockade (5), confirmation of inhibition of adenosine induced coronary vasodilation by alpha adrenergic blocking agents would negate its proposed role in the reactive hyperemia response. The present study was designed to investigate the effect of alpha adrenergic block on the coronary vasodilatory response to infused adenosine and to its nucleotide precursor, ATP.

Methods. Mongrel dogs weighing between 18 and 22 kg were anesthetized with morphine (1 mg/kg sc) and pentobarbital (20 mg/kg iv) and positive pressure breathing was established through an occlusive intratracheal tube. After the left chest was opened and the pericardium incised, heparin (10 mg/kg) was given and the left jugular vein and right common carotid artery were cannulated. Where possible the common left coronary artery was cannulated through its aortic ostium via the left subclavian artery; when this was not feasible the left circumflex branch was directly cannulated just below the origin of the left anterior atrial artery. The cannulated artery was then perfused from an air-pressurized reservoir which was kept filled with blood pumped from the left carotid artery. Coronary perfusion pressure was held constant at a mean pressure of 100 mm Hg by appropriate setting of the air pressure in the chamber. Coronary flow rates were measured by an electromagnetic flow meter probe in series with the cannula tubing. The thoracic aorta was ligated and its proximal portion cannulated and bled into another air-pressurized reservoir; a mean aortic pressure of 100 mm Hg was held constant by the pressurized blood level in this chamber. With these arrangements left ventricular afterload was held constant and the preload consisted primarily of coronary venous blood recirculating to the left heart through the lungs.

Mean aortic pressure and mean coronary perfusion pressure were continuously monitored by pressure transducers and recorded together with the coronary flow curve on a physiological recorder.

Metabolic conditions were held as close to normal as possible by appropriate setting of the respirator and by a continuous slow infusion of 0.2 *M* sodium bicarbonate solution. During each study, a minimum of four coronary arterial blood samples were obtained for determination of pO_2, pCO_2, pH and

[1] Supported by U. S. Public Health Service Grant 5P01HE06304 and the American Heart Association, Northeast Ohio Chapter.

hematocrit.

Solutions of adenosine from commercial sources were made up in physiological saline buffered to pH 7.40 in concentrations of 10, 000 nmoles/ml, and constant rate infusions of from 116 nmoles/min (0.116 ml/min) to 11,600 nmoles/min (1.16 ml/min) were made directly into the coronary cannula tubing. Similarly, solutions of ATP were made up and buffered to pH 7.40 in concentrations of 1000 nmoles/ml, and infused at similar constant rates. Control infusions of saline were made in each animal at these rates.

Coronary flow responses to graded infusion doses of adenosine and ATP were measured before and after alpha adrenergic block. Alpha adrenergic block was produced by phenoxybenzamine, 10 mg/kg, infused iv over a period of 1–2 hr; this regimen was established in other animals with intact peripheral vascular beds where completion of alpha block over this period of time was verified by failure of an arterial pressure response to 50 μg of noradrenaline iv.

Dose calculations were made in terms of the coronary arterial concentrations of adenosine or ATP produced by the different infusion doses by the relationship

$$\text{Concentration (nmoles/100 ml of coronary arterial blood)} = \frac{\text{Infused dose (nmoles)}}{\text{Coronary flow during infusion (ml/min)}} \times 100.$$

Coronary flow responses to the infusions were measured as the ratio of the peak flow rates obtained during infusion to the peak coronary flow rates obtained after 20 sec of coronary occlusion; the latter was produced by clamping the inflow tubing. These reactive hyperemia flow responses were obtained before, during and after each set of infusion studies with an appropriate waiting period to allow return to control flow. The flow response to the graded doses of adenosine and ATP was calculated by the relationship

$$\text{Coronary Flow Response} = \frac{\text{Peak coronary flow rate during infusion}}{\text{Peak coronary flow rate during reactive hyperemia}} \times 100.$$

Statistical evaluation of the effect of adrenergic block on the semilogarithmic dose response curves of increasing concentrations of adenosine and ATP was made in a standard fashion. Regression lines for each set of observations were calculated by the method of least squares, and differences in potency after adrenergic block were tested by a statistical parallel line bioassay of the data obtained from the regression lines of dose responses before and after block (6).

In those animals in which the common left coronary artery was cannulated the weight of the area perfused was obtained by excision of the left ventricle. In left circumflex cannulations the area perfused was estimated as 37% of total heart weight (7).

Results. Table I presents the peak coronary flow rates after 20 sec of coronary occlusion, and the maximal coronary flow rates produced by adenosine and ATP infusion in each animal before and after adrenergic block. These maximal flow rates were produced by the arterial concentrations of adenosine and ATP indicated in Table I which were those beyond which no further increase in flow occurred. Although the percent increase from control of coronary flow in reactive hyperemia, and with adenosine and ATP infusions, was reduced after alpha block, this was due to the increase in the level of the control coronary flow rates induced by the blocking agent rather than a decrease in peak flow. In fact, as shown in Table I, the peak flow rates of both reactive hyperemia and the infusions increased after alpha blockade.

Figures 1 and 2 illustrate the semilogarithmic dose response data for each animal before and after alpha adrenergic block. The average metabolic data during the course of the study is also indicated for each animal.

The four animals illustrated in Fig. 1 showed no consistent change in responsiveness to adenosine after alpha block as calculated from the regression lines and potency ratios. Although one animal (No. 3) showed a reduction in response to adenosine after alpha adrenergic block, the three other animals showed either no change or an enhanced response.

Similarly, in Fig. 2 the dose response regression lines show variation in potency of the ATP infusion before and after alpha adrenergic block among the animals studied; however, only one animal (No. 1) showed a

TABLE I. Comparison of Maximal Flow Rates Obtained by Adenosine and ATP Coronary Arterial Infusions Compared to Peak Reactive Hyperemia Flow.

Animal	Artery	Wt[a] (g)	Condition	Flow (ml/min)		% Increase	Flow (ml/min)		% Increase	Coronary arterial concentration (nmoles/100 ml)
				Control	RH[b]		Control	Infusion		
Adenosine										
No. 1	common	140	Before	160	272	70	160	272	70	4250
			After	224	298	33	240	300	25	3870
No. 2	circ	65	Before	32	82	156	28	58	107	8000
			After	68	106	56	65	98	51	4650
No. 3	circ	63	Before	40	132	230	40	136	241	4288
			After	108	172	64	108	152	41	3840
No. 4	common	106	Before	102	265	160	105	183	74	3180
			After	235	320	36	232	320	38	3640
ATP										
No. 1	circ	68	Before	44	106	141	47	110	134	265
			After	96	126	31	92	117	27	249
No. 2	common	97	Before	90	204	127	93	200	115	145
			After	172	259	51	193	252	31	231
No. 3	circ	63	Before	36	113	214	33	120	264	242
			After	90	137	47	86	126	47	251
No. 4	circ	53	Before	18	83	361	18	86	378	135
			After	73	125	71	68	110	62	264

[a] Wt indicates either total left ventricle (common) or area perfused by circumflex artery (circ).

[b] RH indicates peak reactive hyperemia flow after 20 sec of coronary occlusion.

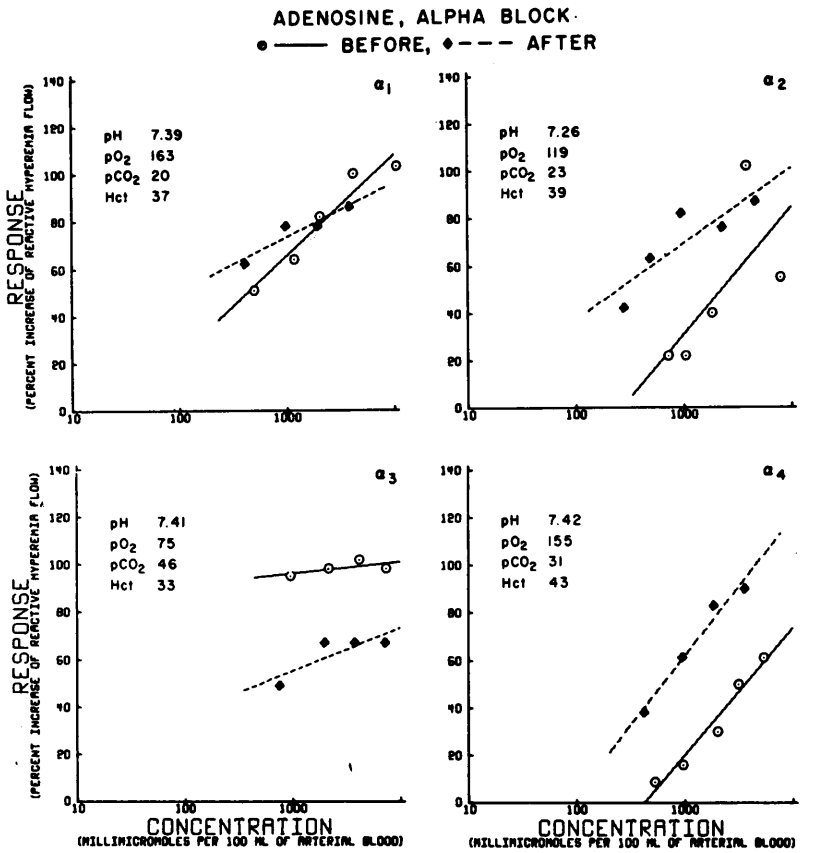

FIG. 1. Effect of alpha adrenergic block on coronary flow response to adenosine. The abscissa (concentration) is logarithmic, the ordinate (response) is linear.

decrease in responsiveness.

Discussion. As expected, blockade of alpha adrenergic receptor sites in the coronary vasculature decreased coronary vascular resistance so that control coronary flow rates following the blockade were consistently higher than before. Thus, the change from control flow induced either by temporary coronary occlusion or by infusions of adenosine or ATP after alpha blockade could not fairly be compared with percentage change from control as a result of similar interventions before the blockade, since the percent increase from control flow would necessarily always be less than before the blockade. Consequently, we compared the vasodilatory potency of adenosine and ATP before and after alpha adrenergic blockade in relation to the maximal vasodilatory capacity of the coronary resistance vessels as reflected by the peak flow rates of reactive hyperemia after temporary coronary occlusion. These reactive hyperemia responses served as the reference flow rates for calculation of responses to adenosine and ATP infusions rather than the resting control flow rates; this method of dose response measurement was used in an earlier study of the coronary vascular effects of adenosine and the nucleotides (8).

The results of the present study fail to confirm the inhibiting effect of alpha adrenergic block on adenosine induced coronary vasodilation as reported by Nayler *et al.* (4) in isolated rat and guinea pig hearts. In addition, the coronary vasodilatory effect of ATP was not consistently decreased by alpha adrenergic block, and the lack of a significant inhibiting effect of alpha adrenergic block on the myocardial reactive hyperemia response to temporary coronary occlusion was confirmed (5). Although the "adenosine hypothesis" as related to myocardial reactive

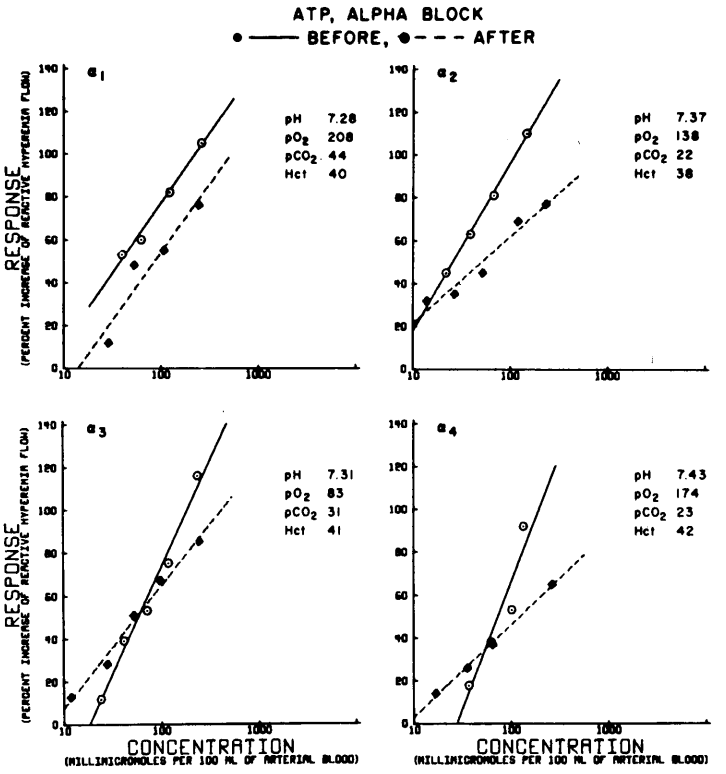

FIG. 2. Effect of alpha adrenergic block on coronary flow response to ATP. Abscissa and ordinate as in Fig. 1.

hyperemia is, therefore, supported to the extent that adenosine (and ATP) induced coronary vasodilation is not effected through inhibition of an alpha adrenergic mechanism, no further information regarding its mode of action was gained from this study. The alternative possibility that adenosine might increase coronary flow by stimulation of beta adrenergic receptor sites in the coronary resistance bed has been excluded by the study of Buckley (9) which showed no inhibition of the adenosine coronary vasodilator effect in beta adrenergic blocked hearts.

As shown in Table I, adenosine infusions resulting in coronary arterial concentrations greatly in excess of those reported by Rubio *et al.* (2) generally failed to cause an increase in coronary flow rates equivalent to those of the peak reactive hyperemia responses to 20 sec of coronary occlusion. In this regard, the results of the present study are similar to those previously reported by us in a comparative study of the coronary vasodilatory effects of adenosine and the nucleotides; possible reasons for the dose response discrepancy for adenosine between the two studies have been discussed (8).

On the other hand, increases in coronary flow rates similar to those of reactive hyperemia were obtained at arterial concentrations of ATP in the range of those reported as giving maximal coronary vasodilation by Wolf and Berne. As previously shown by us (8) and by others (10–12) ATP is considerably more potent as a coronary vasodilator than adenosine. As indicated by the results of this study (Table I), maximal coronary flow responses as judged by increases in coronary flow rates equivalent to that of reactive hyperemia were obtained at low arterial concentrations of ATP, and it is tempting to consider a direct role of this nucleotide in the coronary vasodilatory response to temporary coronary occlusion. Forrester and Lind have reported the presence of ATP in the venous blood from human forearms and demonstrated an increase in its concentration with sustained contracture of the forearm

musculature (13). Additionally, ATP may cross intact cell membranes under other conditions (14–16). Most recently, Chen et al. (17) have documented an increase in ATP concentrations in coronary sinus plasma collected during myocardial hyperemia induced by stellate ganglion stimulation; however, there was no significant change in coronary venous plasma ATP levels during the reactive hyperemia response after temporary coronary occlusion. The source of the ATP and its role as a possible factor in the coronary vasodilatory response to the increase in myocardial oxygen demand induced by sympathetic nerve stimulation remains to be elaborated.

The authors wish to thank Mrs. Jacqueline Rutkowski, Mr. John Dattilo, and Mr. Richard Williams for their assistance in the completion of these studies.

1. Rubio, R., and Berne, R. M., Circ. Res. **25**, 407 (1969).

2. Rubio, R., Berne, R. M., and Katori, M., Amer. J. Physiol. **216**, 56 (1969).

3. Olson, R. W., Circ. Res. **26**, 301 (1970).

4. Nayler, W. G., Price, J. M., and Lowe, T. W., Cardiovasc. Res. **1**, 63 (1967).

5. Pitt, B., Khouri, E. M., and Gregg, D. E., The Physiologist **8**, 251 (1965).

6. Finney, D. J., "Statistical Methods in Biological Assay," p. 99. Griffin, London (1952).

7. Eckstein, R. W., Circ. Res. **5**, 230 (1957).

8. Moir, T. W., and Downs, T. D., Amer. J. Physiol. **222**, 1386 (1972).

9. Buckley, N. M., Amer. J. Physiol. **218**, 1399 (1970).

10. Wolf, M. A., and Berne, R. M., Circ. Res. **4**, 343 (1956).

11. Walter, P., and Bassange, E., Pflügers Arch. **299**, 52 (1968).

12. Winbury, M. D., Papierski, D. H., Hemner, M. D., and Hambourger, W. E., J. Pharmacol Exp. Therap. **109**, 255 (1953).

13. Forrester, T., and Lind, A., Fed. Proc. **28**, 1280 (1969).

14. Boyd, I. A., and Forrester, T., J. Physiol. **199**, 115 (1968).

15. Douglas, W., Poisner, A., and Rubin, P., J. Physiol. **179**, 130 (1965).

16. Holton, P., J. Physiol. **145**, 494 (1959).

17. Chen, W. T., Chou, C. C., Scott, J. B., and Haddy, F. J., The Physiologist **15**, 104 (1972).

Received June 7, 1973. P.S.E.B.M., 1973, Vol. 144.