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CORONARY VASODILATOR RESPONSES TO HYPOXIA BEFORE AND AFTER AMINOPHYLLINE

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SUMMARY

1. In previous studies adenosine has been postulated to be the mediator in coronary blood flow regulation and aminophylline was found to inhibit the coronary vasodilator action of adenosine. The present study was performed to determine whether aminophylline inhibits coronary vasodilatation induced by hypoxia.

2. Coronary and systemic haemodynamic and metabolic effects of hypoxia were studied in anaesthetized dogs before and after aminophylline. Aminophylline did not influence coronary vasodilatation induced by hypoxia.

3. These results are held not to lend support to the hypothesis that adenosine is the metabolite responsible for the regulation of coronary blood flow.

INTRODUCTION

Autoregulation of coronary blood flow is a well-known phenomenon. Many theories have been postulated to explain the intimate mechanism of this phenomenon. The adenosine hypothesis for control of coronary blood flow proposes that adenosine is continuously released by the myocardial cells into the interstitial fluid and its release is enhanced in response to either an increase in cardiac oxygen consumption or a reduction in arterial oxygen supply (Rubio & Berne, 1969). Experimental evidence obtained in recent studies (Rubio & Berne, 1969; Rubio, Berne & Katori, 1969) that adenosine was recovered from coronary sinus blood collected during hyperaemia and in pericardial perfusates under normal and asphyxial conditions supports this hypothesis.

Afonso (1970) and Afonso & O'Brien (1970) found that aminophylline inhibits the coronary vasodilator action of exogenous adenosine administered intravenously or into the coronary artery. It seemed that the attractive adenosine hypothesis could be further validated if coronary vasodi-

lator responses to hypoxia were inhibited by aminophylline. Experiments performed in this study were designed therefore to determine the coronary and systemic haemodynamic and metabolic effects of hypoxia before and after administration of aminophylline.

METHODS

Twelve healthy mongrel dogs averaging 20·9 kg in weight were used to determine the influence of aminophylline on coronary and systemic effects of hypoxia. All animals were anaesthetized with morphine sulphate 3 mg/kg s.c. followed in 1 hr by I.V. administration of allobarbitone 12·5 mg/kg, urethane 50 mg/kg, monoethylurea 50 mg/kg and sodium pentobarbitone 8 mg/kg. A Cournand needle was inserted percutaneously into each femoral artery, one for recording pressure and the other for withdrawal of blood. All animals received 300 μ. heparin/kg at the beginning of the experiments. Cardiac catheters were placed under fluoroscopic control in the right atrium, pulmonary artery and coronary sinus. Coronary sinus blood flow was recorded on a Sanborn Polyviso, as measured by a thermodilution catheter flowmeter (Afonso, 1966) inserted through the jugular vein into the coronary sinus. Blood pressures were recorded with Statham strain gauges on a Gilson Macropolygraph. Cardiac output was calculated from dye dilution curves obtained after injection of indocyanine green into the pulmonary artery. A Stephenson respirator was used to ventilate the animal artificially. The ventilatory rate was kept constant throughout the study. Either room air or a hypoxic gas mixture could be fed from a rubber bag through a three-way stopcock into the respirator.

The study was planned as follows:

In the first part of the study while the dog breathed room air, control measurements were made of heart rate, coronary sinus blood flow, femoral and pulmonary artery and right atrial blood pressures, and simultaneous samples of blood were obtained from the femoral and pulmonary artery and the coronary sinus. A dye dilution curve was obtained for determination of cardiac output. After these measurements were made coronary blood flow was recorded continually and the hypoxic gas mixture was administered. During hypoxia, when coronary flow had stabilized at a higher level, measurements of heart rate, coronary flow, pressures and cardiac output were repeated and blood samples were obtained as indicated.

Aminophylline was then administered in the dose of 7·5 mg/kg into the right atrium. Aminophylline has a coronary vasodilator effect which lasts about 10 min. 20 min after aminophylline was administered and when coronary flow had returned to control level the second part of the study was begun. The same measurements were obtained as in the first part of the study, before and while the animal breathed the hypoxic gas mixture. A mixture of 8 % oxygen in nitrogen was used in seven dogs and 5 % oxygen in nitrogen in five dogs. In seven of these dogs coronary vasodilator responses to adenosine were also obtained before and after aminophylline, in order to measure the inhibition of adenosine induced coronary vasodilatation by aminophylline. In four dogs adenosine was administered into the right atrium as constant rate infusions and in three as single injections.

Left and right ventricular work and total peripheral and pulmonary resistances were calculated by standard formulae. Body oxygen consumption was calculated as the product of cardiac output (l./min) and systemic arterial-pulmonary arterial oxygen difference (ml./l. blood). Analyses of blood oxygen were made by the Van Slyke-Neill method. Coronary sinus blood flow refers to the flow at the cross-section of coronary sinus where the thermistor of the flowmeter was located and may not

represent total coronary sinus blood flow, but since the flowmeter was inserted firmly in the coronary sinus and was not moved during the study it should measure a constant fraction of coronary sinus blood flow. Myocardial oxygen consumption was calculated as the product of measured coronary sinus blood flow (ml./min) and the arterial-coronary sinus oxygen difference (ml./ml. blood). There is no way to determine the amount of myocardial mass to which the calculated myocardial oxygen consumption is related. Therefore values of this parameter are not absolute; however, relative changes in the same experiment represent changes in the oxygen consumption of the left ventricle.

In addition, coronary vasodilator responses to hypoxia and to constant rate infusions of adenosine were determined in a group of seven dogs, in order to verify that the degree of hypoxia used in this study (namely 5 and 8 % oxygen) does not produce maximal dilatation of the coronary vascular bed. The animals were anaesthetized as indicated. A thermodilution flowmeter was placed under fluoroscopic control into the coronary sinus. For infusions of adenosine a cardiac catheter was placed into the right atrium or a no. 5 cardiac catheter with a wire cage of 3 mm diameter affixed at its tip was inserted through the carotid artery and wedged in a branch of the left coronary artery. The animals were ventilated artificially as mentioned. Coronary sinus blood flow was recorded continually while the animal breathed room air and during the administration of the hypoxic gas mixture. After these measurements were obtained, coronary vasodilator responses to constant rate infusions of adenosine were determined while the animal breathed room air. The whole procedure was then repeated. In four animals a hypoxic gas mixture of 5 % oxygen in nitrogen was used and adenosine was infused into the right atrium. In three animals the hypoxic gas mixture contained 8 % oxygen in nitrogen and adenosine was infused into the coronary artery.

Drugs. The aminophylline used was from G. D. Searle & Co. and adenosine (A Grade) from Calbiochem.

RESULTS

Effect of aminophylline on the response to hypoxia

Values of the measured and calculated parameters during hypoxia were compared to their respective control values and analysed statistically by the *t* test for paired values. Changes that occurred during hypoxia, before aminophylline was administered, are shown in Table 1 and after aminophylline in Table 2. Comparable increases in coronary sinus blood flow occurred during hypoxia before and after aminophylline. A representative recording of coronary vasodilator responses to hypoxia before and after aminophylline from one of the dogs is shown in Fig. 1. During hypoxia before aminophylline greater increases occurred in heart rate, cardiac output, femoral arterial blood pressure, and left ventricular work. The control values of the arterial oxygen content compared favourably before and after aminophylline. However, the control coronary sinus oxygen values after aminophylline were lower than the corresponding ones before aminophylline. After aminophylline the coronary sinus oxygen content during hypoxia decreased more (1·8 vol. %) than it did before aminophylline (3·0 vol. %). In four of the dogs coronary vasodilator responses to

TABLE 1. Coronary and systemic haemodynamic effects of hypoxia before aminophylline

Parameter	Control	Hypoxia	% change	P value
Heart rate (beats/min)	82 ± 21	103 ± 28	+25·6	< 0·01
Mean femoral arterial blood pressure (mm Hg)	115 ± 11	132 ± 14	+14·8	< 0·001
Mean pulmonary arterial blood pressure (mm Hg)	13 ± 3	15 ± 3	+15·4	< 0·01
Mean right atrial blood pressure (mm Hg)	4·9 ± 1·5	4·1 ± 1·6	−16·3	< 0·01
Arterial oxygen content (ml./100 ml.)	18·2 ± 2·3	11·9 ± 3·7	−34·8	< 0·001
Mixed venous oxygen content (ml./100 ml.)	13·8 ± 2·8	8·4 ± 2·1	−39·1	< 0·01
(Arterial-mixed venous) oxygen (ml./100 ml.)	4·6 ± 1·5	3·5 ± 1·0	−23·9	0·1 > P > 0·05
Body oxygen consumption (ml./min)	109 ± 21	99 ± 20	−9·2	0·2 > P > 0·1
Cardiac output (l./min)	2·38 ± 0·67	2·95 ± 0·87	+23·9	< 0·01
Left ventricular work (kg-m/min)	3·7 ± 1·1	5·3 ± 1·7	+43·2	< 0·001
Right ventricular work (kg-m/min)	0·5 ± 0·2	0·6 ± 0·3	+20·0	< 0·01
Total peripheral resistance (dynes cm^{-5}/sec)	4203 ± 1208	3906 ± 1265	−7·1	0·3 > P > 0·2
Total pulmonary resistance (dynes cm^{-5}/sec)	478 ± 142	435 ± 166	−9·0	0·1 > P > 0·05
Coronary sinus oxygen content (ml./100 ml.)	6·3 ± 2·0	3·0 ± 1·4	−52·4	< 0·001
(Arterial-coronary sinus) oxygen (ml./100 ml.)	12·0 ± 2·4	8·9 ± 2·7	−25·8	< 0·001
Coronary sinus blood flow (ml./min)	36 ± 8	66 ± 21	+83·3	< 0·001
Cardiac metabolic rate for oxygen (ml./min)	4·3 ± 1·5	5·5 ± 1·3	+27·9	< 0·001
Coronary vascular resistance units (MABP/CBF)	3·41 ± 1·07	2·21 ± 0·79	−35·2	< 0·001

Values are means ± S.D.

TABLE 2. Coronary and systemic haemodynamic effects of hypoxia after aminophylline

Parameter	Control	Hypoxia	% change	*P* value
Heart rate (beats/min)	135 ± 39	157 ± 44	+16·3	< 0·02
Mean femoral arterial blood pressure (mm Hg)	110 ± 20	113 ± 21	+2·7	0·5 > *P* > 0·4
Mean pulmonary arterial blood pressure (mm Hg)	15 ± 3	15 ± 4	—	0·9 > *P* > 0·8
Mean right atrial blood pressure (mm Hg)	4·2 ± 1·3	4·1 ± 1·6	−2·4	0·8 > *P* > 0·7
Arterial oxygen content (ml./100 ml.)	19·0 ± 2·2	12·4 ± 3·2	−34·7	< 0·001
Mixed venous oxygen content (ml./100 ml.)	14·5 ± 1·7	8·9 ± 2·0	−38·6	< 0·001
(Arterial-mixed venous) oxygen (ml./100 ml.)	4·8 ± 1·3	4·1 ± 0·7	−14·6	< 0·05
Body oxygen consumption (ml./min)	112 ± 19	108 ± 20	−3·6	0·3 > *P* > 0·2
Cardiac output (l./min)	2·56 ± 0·54	2·89 ± 0·65	+12·9	< 0·01
Left ventricular work (kg-m/min)	3·9 ± 1·2	4·5 ± 1·6	+15·4	< 0·02
Right ventricular work (kg-m/min)	0·5 ± 0·2	0·6 ± 0·3	+20·0	0·1 > *P* > 0·05
Total peripheral resistance (dynes cm^{-5}/sec)	3577 ± 851	3260 ± 714	−8·9	< 0·02
Total pulmonary resistance (dynes cm^{-5}/sec)	479 ± 136	423 ± 117	−11·7	< 0·01
Coronary sinus oxygen content (ml./100 ml.)	4·5 ± 1·7	1·8 ± 0·9	−60·0	< 0·001
(Arterial-coronary sinus) oxygen (ml./100 ml.)	14·4 ± 2·7	10·6 ± 2·8	−26·4	< 0·001
Coronary sinus blood flow (ml./min)	39 ± 7	68 ± 17	+74·4	< 0·001
Cardiac metabolic rate for oxygen (ml./min)	5·7 ± 1·9	7·0 ± 1·5	+22·8	< 0·01
Coronary vascular resistance units (MABP/CBF)	2·96 ± 0·84	1·75 ± 0·54	−40·9	< 0·001

Values are means ± S.D.

adenosine, administered as constant rate infusions were measured. A representative recording is shown in Fig. 2. Before and after aminophylline, values of coronary blood flow during hypoxia and during adenosine infusions, were compared to respective control ones and the increases expressed as percentages are given in Fig. 3. Before and after aminophylline coronary vasodilator responses to hypoxia were comparable,

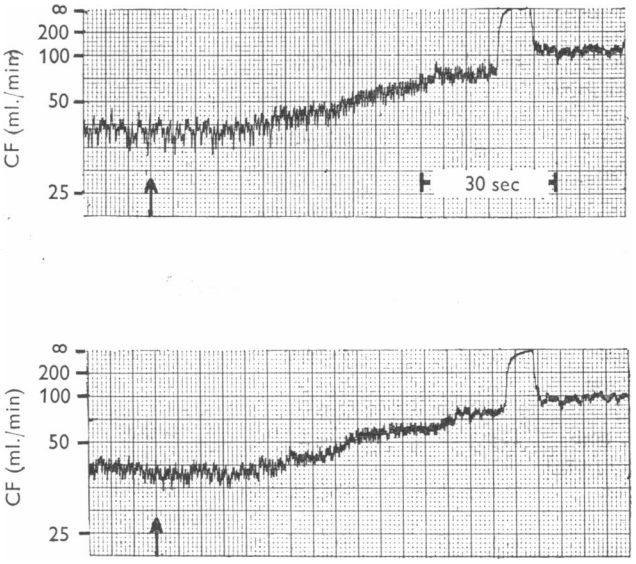

Fig. 1. Shows recordings of coronary sinus blood flow (CF): upper tracing, before aminophylline. At arrow 5 % oxygen in nitrogen was administered. Lower tracing, after aminophylline. Coronary sinus blood temperature was verified in both panels by turning off the heater of the flowmeter (Afonso, 1966).

while after aminophylline adenosine was less effective (2 to 4 times) in inducing coronary vasodilatation than beforehand. The same degree of inhibition of adenosine was observed in the three dogs in which coronary vasodilator responses to adenosine administered as single injections were determined. After aminophylline, 2 mg adenosine produced a response similar to 1 mg adenosine before aminophylline in one dog; the response to 4 mg was similar to that to 2 mg before aminophylline in the second dog; and in the third animal, the response to 2 mg was similar to 0·5 mg adenosine beforehand.

Comparison of responses to hypoxia and adenosine

A representative recording of increases in coronary sinus blood flow produced by hypoxia and adenosine infusions is shown in Fig. 4. Values

of coronary sinus blood flow during hypoxia and during adenosine infusions were compared to respective control values in seven dogs and the increases, expressed as percentages, are shown in Fig. 5. Results from this group of dogs indicate that the degree of hypoxia used in this study (5 and 8 % oxygen) does not produce maximal coronary vasodilatation and the coronary vascular bed is still capable of being further dilated by adenosine administered I.V. or into the coronary artery.

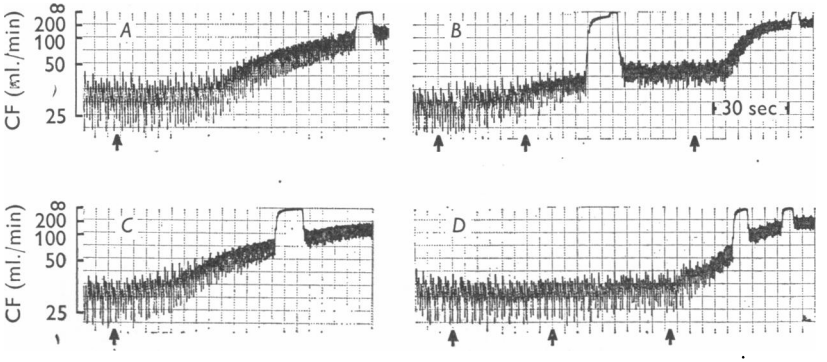

Fig. 2*A*. Coronary vasodilator response to hypoxia before aminophylline. At arrow 5 % oxygen in nitrogen was administered. *B*, coronary vasodilator responses to adenosine, before aminophylline. Arrows indicate time infusions of 3·8, 7·6 and 15·2 mg adenosine/min were started. *C*, coronary vasodilator response to hypoxia after aminophylline. At arrow 5 % oxygen was administered. *D*, coronary vasodilator responses to adenosine after aminophylline. Arrows indicate time infusions of 7·6, 15·2 and 30 mg adenosine/min were started.

Note that before and after aminophylline coronary vasodilator responses to hypoxia are comparable, while coronary vasodilatation to adenosine is inhibited by aminophylline.

Coronary sinus blood temperature was verified in all panels by switching off the heater of the flowmeter (Afonso, 1966).

DISCUSSION

Adenosine has been suggested as the mediator of coronary blood flow regulation (Rubio & Berne, 1969). Aminophylline was found to reduce by 50–75 % coronary vasodilator effects of adenosine administered I.V. or into the coronary artery (Afonso, 1970; Afonso & O'Brien, 1970). Although the intimate mechanism of this inhibitory action is not known, it has been suggested that aminophylline might act on the myocardium or coronary vascular smooth muscle to produce this effect. The current study was undertaken to examine the effect of aminophylline on the coronary vasodilatation induced by hypoxia in the expectation that a decreased coronary vasodilator response to hypoxia after aminophylline would sup-

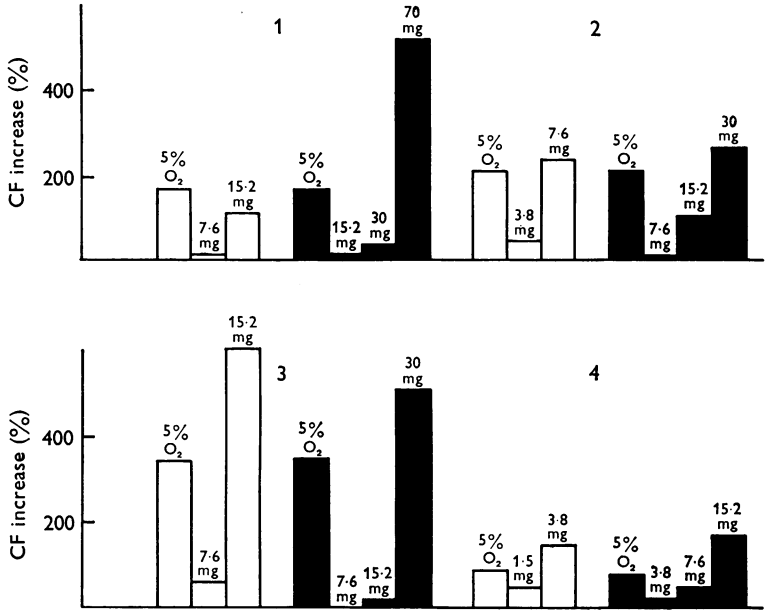

Fig. 3. CF, coronary sinus blood flow. Coronary sinus blood flow increases produced by hypoxia and adenosine, before and after aminophylline in four dogs. Open columns, increases before aminophylline. Shaded columns, increases after aminophylline. Numbers above columns represent oxygen concentration of hypoxic gas mixture and dose of adenosine/min administered as constant rate infusions into the right atrium.

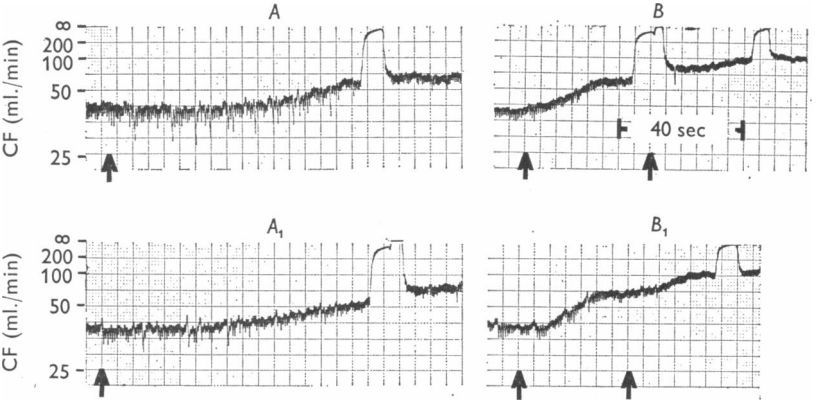

Fig. 4. Recordings of coronary sinus blood flow: *A*, coronary vasodilator response to hypoxia. At arrow 5 % oxygen in nitrogen was administered. A_1, respective repeat. *B*, coronary vasodilator response to adenosine infusions administered into right atrium. Arrows represent time infusions of 3·8 mg/min and 7·6 mg/min started. B_1, respective repeat. Coronary sinus blood temperature was verified in all panels by switching off the heater of the flowmeter (Afonso, 1966). Note that 7·6 mg/min of adenosine produced a greater response than that to 5 % oxygen.

port the adenosine hypothesis of coronary flow regulation. Results show that coronary blood flow increases produced by hypoxia before and after administration of aminophylline are of the same magnitude. Although the increase was 83% before aminophylline as compared to 74% after aminophylline, this difference can be easily explained on the basis of

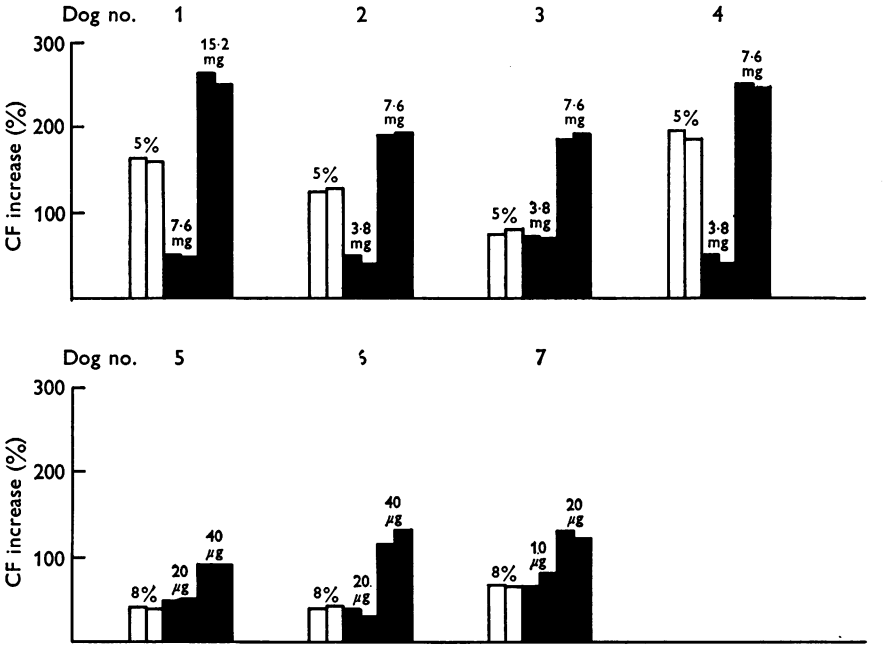

Fig. 5. CF, coronary sinus blood flow. Coronary sinus blood flow increases produced by hypoxia and adenosine. Open columns, increases produced by hypoxia. Numbers above columns represent oxygen concentration of the hypoxic gas mixture. Shaded columns, increases produced by constant rate infusion of adenosine. Numbers above indicate dose of adenosine/min used. In dogs nos. 1–4 adenosine was infused into right atrium and nos. 5–7 into coronary artery. One of each pair of columns is a duplicate determination.

greater increases in heart rate and left ventricular work which occurred during hypoxia before the administration of aminophylline. These results do not provide support to the attractive adenosine hypothesis of coronary flow regulation. In making this interpretation we considered the possibility that if the degree of hypoxia used leads to a maximal dilatation then endogenous adenosine might be present in supramaximal concentration. Hence even if the potency of exogenous adenosine were reduced by aminophylline there might be no diminution in the response to hypoxia though that to submaximal doses of injected adenosine would be reduced. This

possibility however can be ruled out by the finding that the degree of hypoxia used (5–8 % oxygen) does not produce maximal coronary vasodilatation and the coronary vascular bed can be further dilated by adenosine, administered I.V. or into the coronary artery.

In view of the negative findings it is reasonable to question whether they invalidate the adenosine hypothesis or merely fail to support it. In this respect, some other findings of this study should be taken into consideration. After aminophylline the values of coronary sinus blood oxygen content are lower than the respective ones before aminophylline both before and during hypoxia. If the rate of formation of endogenous myocardial adenosine is related to the myocardial oxygen tension then it is conceivable that after aminophylline an adjustment could have occurred in which more adenosine was produced at lower myocardial oxygen tension but its coronary vasodilator action was inhibited by aminophylline. If this were the case then the findings of this study would not necessarily invalidate the adenosine hypothesis. On the other hand, if the values of coronary sinus oxygen before and during hypoxia after aminophylline had been comparable to the respective values before aminophylline, then the results of this study could be interpreted as invalidating the adenosine hypothesis. In a previous study (Afonso, 1969) it was found that lidoflazine, an agent which enhances the coronary vasodilator action of exogenous adenosine did not enhance coronary vasodilator responses to hypoxia and induced tachycardia. Unfortunately, no determinations of coronary sinus blood oxygen content were obtained in this study. It is possible that after lidoflazine an adjustment could have occurred in which at higher myocardial oxygen tension less adenosine was produced, but its coronary vasodilator action would be enhanced by lidoflazine.

In summary the results of this study do not provide support for the adenosine hypothesis of coronary blood flow regulation. For the adenosine hypothesis to be considered valid even though the coronary vasodilator response to hypoxia is unaltered by aminophylline or lidoflazine, appropriate variation in the coronary sinus content of adenosine must be shown after lidoflazine and aminophylline.

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