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THE ACTION OF AMINOPHYLLINE ON THE ACUTELY TRANSPLANTED DOG HEART: EFFECT OF α- AND β-ADRENOCEPTOR BLOCKADE

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1 Aminophylline inhibits the coronary vasodilator actions of adenosine. Our previous studies suggested that low dose infusions of aminophylline reduce coronary blood flow in the isolated heart. In the present study we investigated the actions of aminophylline on coronary blood flow and myocardial contractility in a transplanted heart model. Drugs were given by close coronary arterial infusion.

2 Aminophylline in low doses (200 μg/min) reduced coronary blood flow by 21 ± 2% (mean ± s.e. mean) but did not alter myocardial contractility or heart rate. Higher doses (500 and 1000 μg/min) increased coronary blood flow and myocardial contractility without changing heart rate.

3 α-Adrenoceptor blockade with phenoxybenzamine did not affect the response to a low dose of aminophylline (200 μg/min).

4 Propranolol in doses of 10 and 30 μg/min blocked β-adrenoceptors but did not change coronary blood flow. The higher dose reduced myocardial contractility.

5 The effects of a high dose of aminophylline (1000 μg/min) on coronary blood flow were not changed by either α- or β-adrenoceptor blockade, although propranolol (30 μg/min) reduced the augmentation in myocardial contractility.

6 The results show that when given in doses which do not alter myocardial contractility, aminophylline reduces coronary blood flow in the isolated heart and that this is not mediated through an α-adrenoceptor mechanism, They also show that the increases in coronary blood flow and positive inotropic effects obtained with higher doses of aminophylline are not mediated through catecholamines and suggest that higher doses of aminophylline have a small direct coronary vasodilator action. The low dose vasoconstrictor response may be produced by inhibition of the coronary vasodilator action of locally produced adenosine.

Introduction

Aminophylline is widely used in the treatment of bronchial airways obstruction and cardiac failure. The active component theophylline, in combination with ethylenediamine to enhance solubility, produces therapeutic effects largely through its actions on smooth muscle and cardiac muscle (Goodman & Gilman, 1970).

The mechanisms are only partly understood and are controversial (Epstein, Levey & Skelton, 1971). Aminophylline has been reported to have a coronary vasodilator action in the intact heart of both man (Starr, Gamble, Margioles, Donal, Joseph & Eagle, 1937; Howarth, McMichael & Sharpey-Schafer, 1947; Charlier, 1961) and the dog (Stoland, Ginsberg, Loy & Hiebert, 1934; Boyer & Green, 1941; Boyer, 1943) and to increase the energy requirements and contractile state of the heart (Melville & Lu, 1950; Amsterdam, Zelis, Spann, Miller & Mason, 1970). There is evidence that aminophylline influences calcium exchange in the heart (Nayler, 1963; Nayler & Hasker, 1966; Nayler, 1967; McNeill, Nassar & Brody, 1969; Blinks, Olson, Jewell & Braveny, 1972; Massingham & Nasmyth, 1972), that it increases intracellular cyclic adenosine-3,5′-monophosphate (cyclic AMP) through inhibition of phosphodiesterase (Butcher & Sutherland, 1962; Sutherland, Robison & Butcher, 1968) and that it releases catecholamines in the heart (Westfall & Fleming, 1968; Marcus, Skelton, Grauer & Epstein, 1972) and potentiates their effects on blood vessels (Rall & West, 1963; Bartelstone, Nasymth & Telford, 1967; Massingham, 1969; Kalsner, 1971). An additional

direct effect has also been proposed (Marcus *et al.*, 1972; Cohen, Lesne, Valette & Wepierre, 1970).

Our interest in aminophylline resulted from the observation in the transplanted heart that low dose infusions of aminophylline reduced coronary blood flow and increased coronary vascular resistance (Paoloni & Wilcken, 1971, 1972). The aminophylline had been given as part of a study of its inhibition of the coronary vasodilator action of adenosine and other coronary vasodilators. The present study was undertaken to determine the effect of aminophylline on both the coronary blood flow and the contractile state of the transplanted heart and to investigate the role of catecholamines in the aminophylline response by the use of selective adrenoceptor blockade.

Methods

Experimental model

A dog heart (the donor heart) was transplanted into the neck of a second dog (the recipient dog) as described previously (Paoloni & Wilcken, 1971; 1972). The donor heart (weight 90-130 g) was transplanted into the neck of the recipient dog by anastomosing the subclavian artery to the proximal end of the recipient's carotid artery whilst the donor's left pulmonary artery was anastomosed to the recipient's external jugular vein. The donor heart continued to beat forcefully in sinus rhythm throughout the procedure. In this preparation, blood flows into the donor aorta from the recipient's carotid artery at a pressure equal to the recipient dog's systemic pressure. As the superior and inferior venae cavae and azygous veins of the donor heart are ligated, the coronary venous blood of the donor heart is returned to the recipient's circulation through the left pulmonary artery to the external jugular vein anastomosis.

The coronary blood flow (CBF) in this preparation is the volume of blood per unit time which enters the donor aorta from the recipient's carotid artery, provided that the aortic valve is competent (Paoloni & Wilcken, 1971). To measure CBF, an electromagnetic flow probe (IVM Model FT-2HS) was placed on the feeding carotid artery proximal to the anastomosis and connected to an EMI Electromagnetic Flowmeter (Type 28) as previously described (Paoloni & Wilcken, 1971). The perfusion pressure in the donor aorta was measured through a triple lumen catheter (Polyvinyl o.d. 2.50-i.d. 0.96 mm each) inserted through the innominate artery, positioned above the aortic valve and connected to a Statham Pressure Transducer (Model P23.DB). This catheter was also used for drug infusions. The donor electrocardiogram (ECG) was recorded from myocardial electrodes attached to the left ventricle.

The donor left ventricular pressure was measured from a 5 cm long stiff-walled catheter (Bardic Polyvinyl Chloride Angiocath 14 Gauge, o.d. 2.00 mm-i.d. 1.5 mm) introduced transmurally into the left ventricular cavity through the apex. This catheter was connected directly to a Statham P23.DB Pressure Transducer. The dynamic response of the complete catheter/transducer system was checked with a sine wave pressure generator (Gersh, Hahn & Prys-Roberts, 1971; Nejad, Klein, Mirsky & Lown, 1971). When tested in this manner the system had an amplitude/frequency response flat (±5%) to 35 Hz. The ECG and pressure and flow signals were displayed on an oscilloscope screen and recorded on photographic paper with an Electronics for Medicine DR8 Multichannel Recorder. The left ventricular pressure and its electronically derived first derivative (dP/dt) were recorded at a paper speed of 200 mm/s for accuracy of measurement. The dP/dt was measured directly from the left ventricular pressure trace, the electronically derived first derivative (dP/dt) being used only as a guide to the point in the trace at which peak dP/dt occurred. The left ventricular Isometric Tension Time Index (dP/dt^{-1} IIT^{-1}) (Siegel & Sonnenblick, 1963; Siegel, Sonnenblick, Judge & Wilson, 1964; Siegel, 1969) was calculated by integrating (by planimetry) the left ventricular pressure trace from the end-diastolic point to the point of peak dP/dt to obtain the Integrated systolic Isometric Tension (IIT) value and dividing the measured peak dP/dt by IIT. Thus developed pressure was used in the calculation of IIT, not total pressure.

The CBF was measured as the area (by planimetry) under the flow signal per unit of time and converted to ml per min from the direct flow measurement and the calibration signal (Paoloni & Wilcken, 1971). The coronary vascular resistance was estimated as mean donor aortic pressure/CBF. The right atrium of the donor heart was observed to be collapsed during the studies and right atrial pressure was therefore not measured.

There were 20 successful preparations; studies were not performed on any preparation displaying cardiac irregularities or left ventricular distension. The preparations remained stable as regards perfusion pressure, coronary blood flow and arterial blood gases and pH for at least 6 h (Paoloni & Wilcken, 1971).

When control tracings taken between 30 and 60 min after transplantation were found to be steady, drug studies were begun. To avoid the effects of any transient minor changes in CBF each drug-induced alteration in CBF was compared

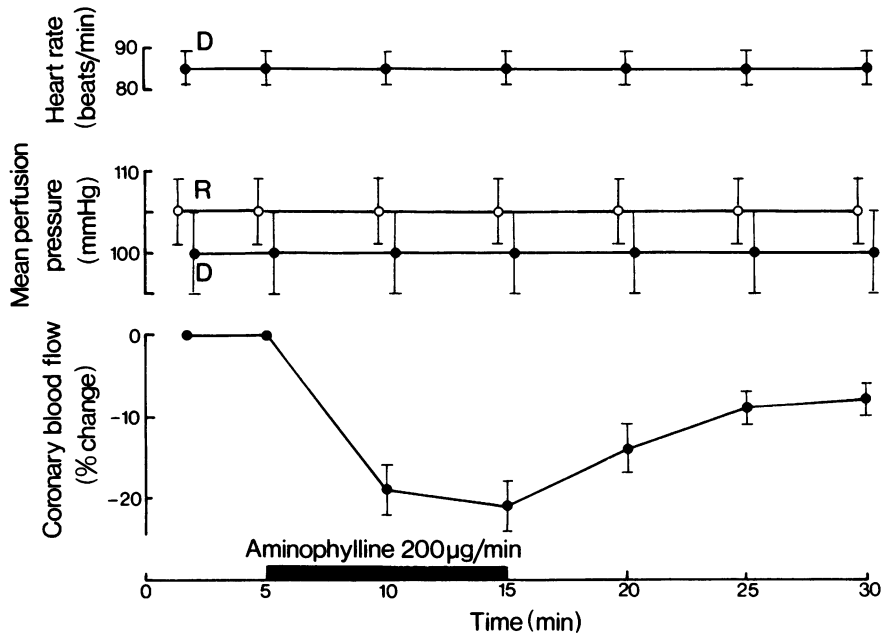

Figure 1 Grouped data from 12 experiments in 12 dogs, showing the changes in coronary blood flow (mean ± s.e. mean) produced by 10 min infusions of aminophylline 200 μg/minute. There were no changes in donor heart rate (D) or in recipient (R) and donor (D) mean aortic pressures.

to a 1 min control flow immediately preceding the drug study. At the end of the studies, each heart remained in sinus rhythm without ectopic beats. There was no cardiac distension.

Drug studies

The drugs in 0.9% w/v NaCl solution (saline) were infused at 1 ml/min (Braun Infusion Pump) into the aorta of the donor heart via the triple lumen catheter whose tip was above the aortic valve. Allowance was made for a catheter transit time of 30 seconds.

In the analysis of the results, Student's *t* test was used to determine the significance of differences between paired observations in each series of experiments.

Results

In all preparations the CBF was found to be steady at 30 to 60 min after transplantation. At 60 min mean CBF (±s.e. mean) was 81 ± 4 ml min^{-1} 100 g^{-1} (range 66 to 106 ml). At the conclusion of each 4 to 6 h study, resting flow had not decreased by more than 10%.

Aminophylline

In each experiment an infusion of aminophylline 200 μg/min into the donor heart reduced CBF. The decrease began within 1 to 2 min of starting the infusion and CBF was at its lowest value after 5 minutes. The grouped responses obtained from 12 experiments in 12 dogs are shown in Figure 1. The CBF was reduced by 19.3 ± 2.5% at 5 min and 21.5 ± 2.7% at 10 min without any change in donor heart rate or perfusion pressure ($P > 0.05$) and no significant alteration in the indices of ventricular contractility (dP/dt, $dP\ dt^{-1}\ IIT^{-1}$) ($P > 0,05$) (Figure 2). On stopping the infusion at 10 min the CBF returned towards, but did not invariably reach, pre-infusion control values (Figure 1). There was, however, a significant increase in CBF 10 min after stopping the infusion ($P < 0.0005$).

In order to determine the influence of ethylenediamine in the low dose aminophylline response, an infusion of ethylenediamine 30 μg/min was given separately, this representing the proportion of ethylenediamine present in the aminophylline 200 μg/min infusion. In 5 experiments in 5 preparations, a 5 min infusion of ethylenediamine did not significantly alter CBF or

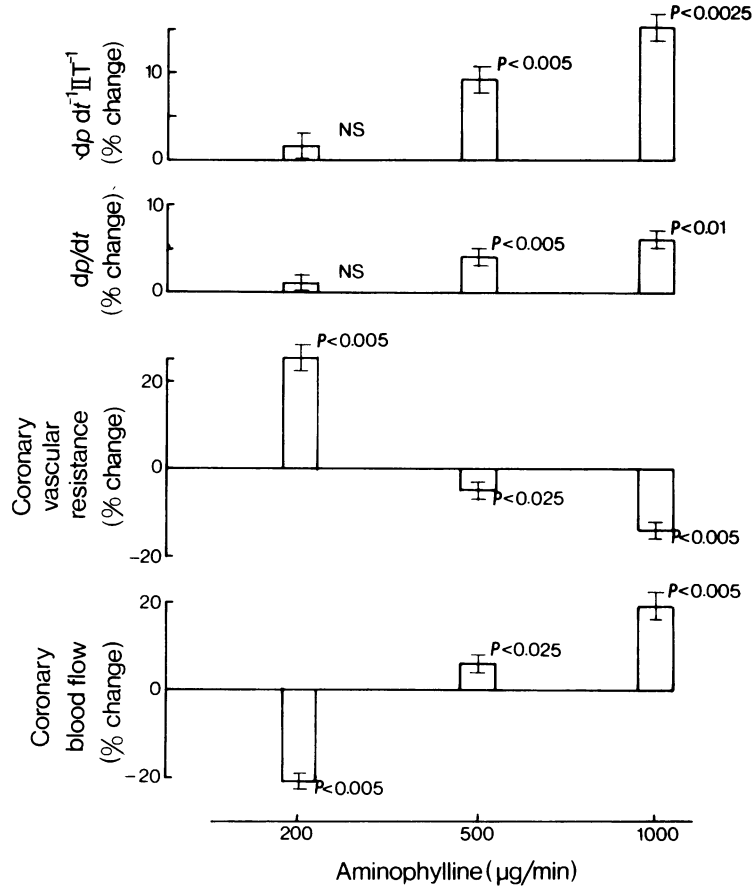

Figure 2 The changes (group data, mean ± s.e. mean) in coronary blood flow, coronary vascular resistance and myocardial contractility (dP/dt dP dt^{-1} IIT^{-1}) produced by 10 min infusions of aminophylline 200 μg/min (10 experiments), 500 μg/min (6 experiments) and 1000 μg/min (6 experiments). There were no significant changes in donor heart rate or perfusion pressure.

Table 1 Changes in coronary blood flow produced by 10-min infusions of aminophylline

Aminophylline	n	*Coronary blood flow (ml min⁻¹ 100 g⁻¹)*		*Coronary blood flow (% change)*	P
		Control	*Response*		
200 μg/min	12	78.6 ± 6.0 (55-107)	62.8 ± 5.5 (35-96)	−21.5 ± 2.7	<0.005
500 μg/min	6	83.4 ± 4.5 (66-106)	88.9 ± 6.0 (68-120)	+6.2 ± 2.0	<0.025
1000 μg/min	6	82.0 ± 6.8 (66-106)	97.8 ± 9.7 (72-130)	+19.4 ± 4.5	<0.005

Measurements are mean with s.e. mean. Figures in parentheses indicate range.

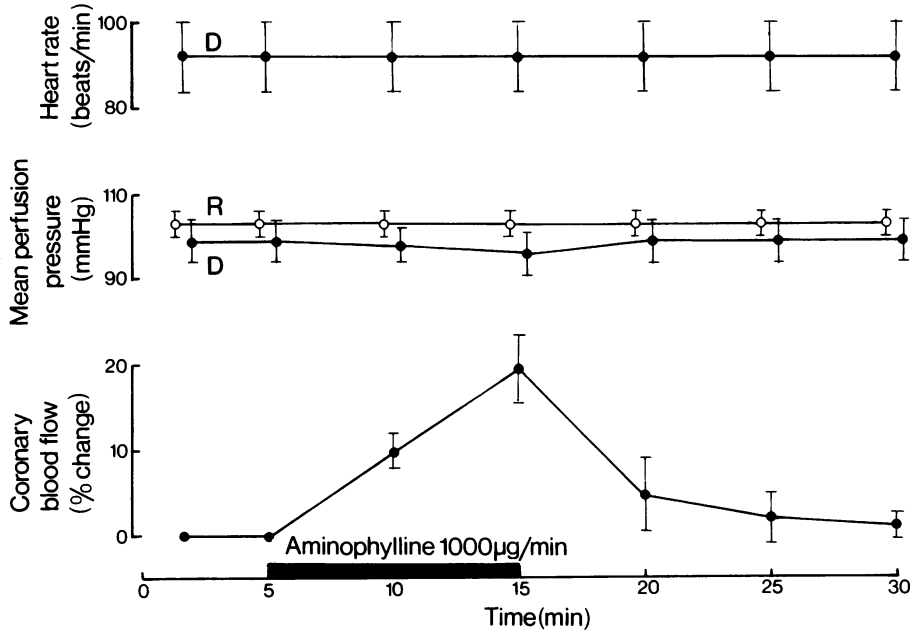

Figure 3 Changes in coronary blood flow (mean ± s.e. mean), recipient (R) and donor (D) mean perfusion pressure and donor heart rate produced by 10 min infusions of aminophylline 1000 μg/min (6 experiments in 6 dogs).

coronary vascular resistance, suggesting that the observed aminophylline responses were due to the theophylline component alone.

A higher dose infusion of aminophylline (1000 μg/min) increased CBF in every experiment. The combined results from 6 experiments in 6 dogs (Figure 3) show an increase in CBF at 5 min of 9.8 ± 2.0% and at 10 min of 19.4 ± 4.5% when the infusion was stopped. The CBF returned to pre-infusional control values by 10 to 15 minutes. There were no significant changes in donor heart rate or perfusion pressures, although both dP/dt and $dP\ dt^{-1}\ IIT^{-1}$ were significantly increased ($P < 0.005$) (Figure 2). In a further 6 experiments, a 10 min infusion of aminophylline 500 μg/min produced an increase in CBF of 6.2 ± 2.0% with a significant increase in dP/dt and $dP\ dt^{-1}\ IIT^{-1}$ ($P < 0.05$) (Figure 2 and Table 1).

Aminophylline and α-adrenoceptor blockade

The mechanism of the aminophylline-induced decrease in CBF was investigated 60 min after premedication of the recipient dog with phenoxybenzamine (2 mg/kg intravenously). The phenoxybenzamine reduced the recipient dog's mean aortic pressure by 16 ± 3 mmHg (mean ± s.e. mean) over this period. The degree of α-adrenoceptor blockade produced by phenoxybenzamine was assessed by giving rapid intravenous injections of 30 μg of phenylephrine to the recipient dog and recording the increase in the recipient dog's mean aortic pressure. Before phenoxybenzamine the increase was 8.0 ± 0.8 mmHg ($P < 0.01$), whereas after phenoxybenzamine, phenylephrine had no significant effect. In 5 preparations after phenoxybenzamine, aminophylline 200 μg/min decreased CBF to a similar extent as in the control studies (Figure 4). In 2 experiments in 2 dogs, aminophylline 1000 μg/min was infused after phenoxybenzamine. The usual increases in CBF, dP/dt and $dP\ dt^{-1}\ IIT^{-1}$ were obtained. In one experiment, the phenoxybenzamine was slowly infused directly into the donor heart. The responses to both aminophylline 200 μg/min and 1000 μg/min were unchanged 1 h after the infusion.

Aminophylline and β-adrenoceptor blockade

The effect of β-adrenoceptor blockade on the aminophylline responses was investigated with propranolol. A 5 min infusion of propranolol 30 μg/min did not significantly change CBF, donor heart rate or perfusion pressure (Paoloni &

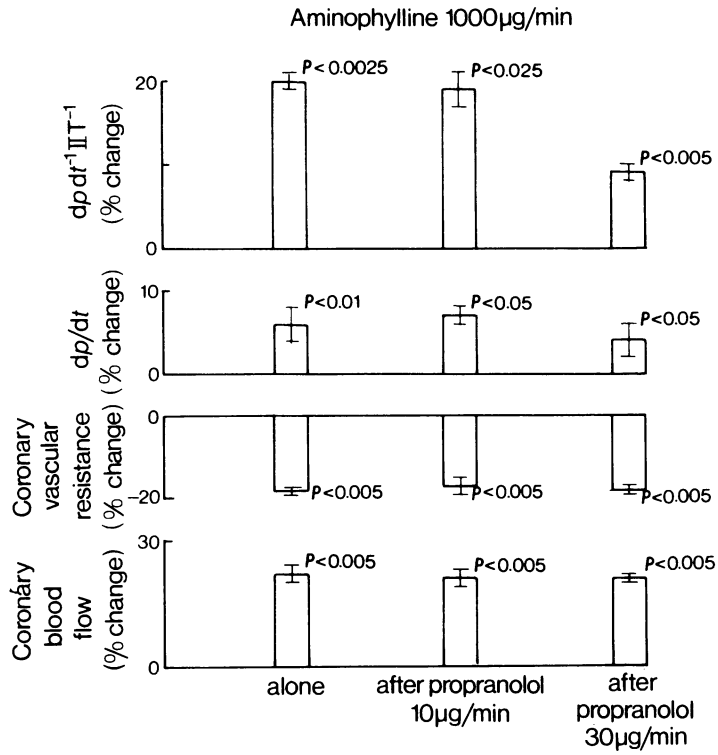

Figure 4 The changes in coronary blood flow (mean ± s.e. mean), coronary vascular resistance and myocardial contractility (d*P*/d*t*, d*P* dt^{-1} IIT^{-1}) produced by 10 min infusions of aminophylline 1000 μg/min, when given alone (10 experiments), after propranolol 10 μg/min (5 experiments) and after propranolol 30 μg/min (5 experiments). The control data comprise controls from both sets of experiments.

Wilcken, 1971), yet fully prevented the increases in CBF and donor heart rate produced by 2 min infusions of isoprenaline 0.5 μg/min given into the donor aorta. This effect was maintained for at least 60 minutes. Propranolol 30 μg/min produced a small negative inotropic effect on the transplanted heart and reduced left ventricular d*P*/d*t* and d*P* dt^{-1} IIT^{-1} by 4 ± 1 and 13 ± 2% respectively.

When aminophylline 1000 μg/min was infused in 5 preparations after an infusion of propranolol 30 μg/min the increases in CBF were not significantly different from those obtained before β-receptor blockade ($P > 0.05$). However, the contractility changes produced by the high dose of aminophylline were decreased after this dose of propranolol ($P < 0.125$) (Figure 4). On repeating the experiments in 5 dogs with a lower, though still a β-receptor blocking, dose of propranolol (10 μg/min) the aminophylline-induced increases in CBF and contractility were not altered, showing that β-receptor blockade with propranolol did not modify the actions of aminophylline 1000 μg/minute.

To check the sensitivity of the left ventricular d*P*/d*t* and d*P* dt^{-1} IIT^{-1} measurements as indices of changes in myocardial contractility, the effects of isoprenaline and ouabain on d*P*/d*t* and d*P* dt^{-1} IIT^{-1} were compared with aminophylline. Infusions of isoprenaline 0.5 μg/min increased d*P*/d*t* and d*P* dt^{-1} IIT^{-1} by 80 ± 5% and 200 ± 50% (mean ± s.e. mean) respectively. Intravenous injections of 0.75 mg of ouabain given to the recipient dog increased the d*P*/d*t* of the donor heart by 9 ± 3% and the d*P* dt^{-1} IIT^{-1} by 19 ± 3% within 15 min without altering the donor heart rate. The mean blood pressure of the recipient dog was however increased by 9 ± 1 mmHg following the ouabain injection.

Discussion

With the methods used, we were able to transplant a beating heart which was perfused continuously

and was not subjected to an anoxic period. The preparation seemed suitable for the study of the effects of vasoactive drugs on the coronary circulation of the acutely transplanted and denervated heart (Paoloni & Wilcken, 1971; 1972).

The methods used to assess changes in myocardial contractility (dP/dt and $dP\,dt^{-1}\,IIT^{-1}$) were based on the studies of Siegel & Sonnenblick (1963), Siegel *et al.* (1964), and Siegel (1969), measurements being made during the isometric phase of left ventricular contraction (see methods section). The accuracy is critically dependent on the fidelity of the pressure recording system. The frequency/response characteristics of the left ventricular catheter/transducer system we used were flat (+5%) to 35 Hz. As the resting donor heart rate was always less than 120/min (usually about 90/min) the pressure recordings were of sufficient fidelity to represent accurately the 15th to 20th harmonic of the pressure pulse. This is adequate for the measurement of peak dP/dt (McDonald, 1960; Gersh *et al.*, 1971). In support of this, the indices we used reflected appropriately not only the gross contractility changes which isoprenaline is known to produce but also the smaller changes after ouabain which were of approximately the same order of magnitude as those we found with aminophylline. Further, during the aminophylline studies the donor heart rates, donor aortic pressures and donor left ventricular end-diastolic pressures remained steady so that the left ventricular pre-load and after-load should not have been altered (Grabner, Conti, Lappe & Ross, 1972). Under these conditions the indices derived from the left ventricular pressure trace which we used appeared suitable for the assessment of changes in myocardial contractility produced by infusions of aminophylline.

Our studies showed that aminophylline given in low doses (200 μg/min) which did not alter myocardial contractility or heart rate, reduced CBF and produced coronary vasoconstriction. When high doses of aminophylline (1000 μg/min) were infused, both CBF and myocardial contractility were increased. There were no changes in donor heart rate. With aminophylline doses of 500 μg/min, the changes were similar to those found with 1000 μg/min but were of smaller magnitude. The doses of aminophylline delivered to the coronary circulation of the transplanted heart were of a similar order to those used clinically in the treatment of bronchial airways obstruction or congestive cardiac failure. Assuming that there is a simple dilution of the drug in the circulating blood volume, an intravenous injection of 250 to 500 mg of aminophylline would initially result in about 1000 to 2000 μg/min being delivered to the coronary circulation. This would represent the upper limit of therapeutic blood levels.

To explain the reduction in CBF produced by low dose infusions of aminophylline, we suggested in an earlier study (Paoloni & Wilcken, 1971) that aminophylline may be potentiating α-adrenoceptor mediated effects of catecholamines on coronary vessels leading to coronary vasoconstriction, since aminophylline will potentiate the pressor effects of catecholamines in the peripheral circulation (Bartelstone *et al.*, 1967). The present studies do not support this as there was no change in the low dose aminophylline vasoconstrictor response after α-receptor blockade. But the response might be explained by low dose infusions of aminophylline inhibiting the vasodilator action of adenosine released from the heart (Afonso, 1970a; Paoloni & Wilcken, 1971). Adenosine is a known powerful coronary vasodilator (Wolf & Berne, 1956; Bretschneider, Frank, Bernard, Kochsiek & Scheler, 1959; Afonso, 1970b; Paoloni & Wilcken, 1971, 1972) and may be involved in the physiological regulation of coronary blood flow (Berne, 1963, 1964). Our earlier studies showed that infusions of aminophylline 200 μg/min will produce marked inhibition of the coronary vasodilator action of adenosine in this preparation (Paoloni and Wilcken, 1971). Thus, whilst we have no direct proof that adenosine blockade is responsible for the aminophylline-induced reduction in CBF, the results are at least consistent with the hypothesis that adenosine release from the heart is important for the regulation of coronary blood flow.

The role of β-adrenoceptors in the control of CBF has been studied by Parratt & Grayson (1966) and Parratt (1967) who showed that β-receptor blockade with propranolol decreased CBF in the intact dog heart. They suggested that there was normally a β-receptor mediated coronary vasodilator effect on the coronary circulation and that propranolol had blocked this. In the transplanted heart, we found no change in resting CBF after propranolol in this study and in a previous one (Paoloni & Wilcken, 1971) as also did Boake & Folts (1971) using a similar preparation. Presumably the β-adrenoceptor mediated vasodilator stimulus was removed by denervation at the time of transplantation. In the present studies, the larger dose of propranolol (30 μg/min) produced a small negative inotropic effect due either to the inhibition of β-receptor stimulation by circulating catecholamines (Davis, MacDonald & Mason, 1969) or to a direct depressant effect on the transplanted heart or to both (Whitsitt & Lucchesi, 1967). When the lower dose of propranolol (10 μg/min) was used there was no significant change in myocardial contrac-

tility although the β-receptor blocking action was retained. These findings suggest that the negative inotropic effect of the higher dose of propranolol was due to a direct depression of myocardial contractility.

Infusions of aminophylline 1000 μg/min resulted in coronary vasodilatation and an increase in myocardial contractility as assessed by dP/dt and $dP\ dt^{-1}\ IIT^{-1}$. The increase in CBF was not influenced by either the high (30 μg/min) or the low (10 μg/min) dose of propranolol. However, the higher dose of propranolol which depressed myocardial contractility also decreased the aminophylline-induced augmentation of myocardial contractility; but despite this, the CBF response was not affected. The lower dose of propranolol produced β-receptor blockade yet did not change contractility or alter the contractility effects of aminophylline. We concluded therefore that β-receptor blockade did not influence the myocardial actions of aminophylline; and that the results with the different doses of propranolol indicated a dissociation between the positive inotropic action of high doses of aminophylline and the coronary vasodilatation which accompanies it, suggesting that in these doses aminophylline has a small direct coronary vasodilator action.

The findings with β-receptor blockade are in agreement with those of both Cohen *et al.* (1970) in the rat heart and Massingham & Nasmyth (1972) in the frog heart, who found that the positive inotropic effects of aminophylline were not influenced by β-receptor blockade with propranolol. However, Marcus *et al.* (1972) produced β-adrenoceptor blockade in the cat isolated papillary muscle with non-depressant doses of propranolol and reported that this reduced the maximum positive inotropic action of theophylline by 40%. These differences in results cannot be explained at present.

Our results show that when given in a dose which does not alter myocardial contractility, aminophylline reduces CBF and increases coronary vascular resistance in the dog isolated heart and that the response is not mediated through an α-adrenoceptor mechanism. They show further that the increases in CBF, the reductions in coronary vascular resistance and the positive inotropic effects obtained with higher doses of aminophylline are not mediated through β-adrenoceptors. The mechanisms of these effects were not identified in the present studies but presumably the known actions of aminophylline on phosphodiesterase (Sutherland *et al.*, 1968) and calcium transport in cardiac muscle (Nayler, 1967) could have been responsible for the high dose effects; the low dose response could have been produced by inhibition of the vasodilator action of locally produced adenosine.

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