Effect of aminophylline on coronary reactive hyperaemia following brief and long occlusion periods

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SUMMARY The effects of an intracoronary aminophylline infusion, adjusted to give a constant concentration of 25 µg·cm⁻³ coronary blood, on the reactive hyperaemic responses following coronary occlusion for 4, 10, and 25 heart beats were investigated in anaesthetised, open-chest dogs. The vasodilator effect of intracoronarily-administered adenosine and the hyperaemic response after coronary occlusion for 10 and 25 heart beats were both significantly diminished under the influence of aminophylline. However, the decrease in the coronary dilator effect of adenosine amounted to 80%, whereas the hyperaemic response was diminished by only 20%. The hyperaemic response following a coronary occlusion for only 4 heart beats remained unchanged. The present results obtained with aminophylline suggest at least a partial involvement of adenosine in mediating reactive hyperaemia after sufficiently long periods of coronary artery occlusion.

The adenosine hypothesis of metabolic regulation of coronary blood flow, firstly proposed by Berne (1963), has been repeatedly supported in the following years (Jagenau and Schaper, 1969; Rubio and Berne, 1969; Rubio et al., 1969; Olsson, 1970; Raberger and Kraupp, 1971; Fox et al., 1974). However, the findings obtained with aminophylline, which is known to inhibit the vasodilator action of exogenous adenosine (Afonso, 1970; Schaumann et al., 1970), are in disagreement with this concept. Aminophylline does not influence coronary dilation induced by hypoxia (Afonso et al., 1972; Wadsworth, 1972) and its effects on coronary post-occlusion hyperaemia are controversial (Juhran and Dietmann, 1970; Juhran et al., 1971; Bittar and Pauly, 1971; Curnish et al., 1972; Wadsworth, 1972; Eikens and Wilcken, 1973a; Eikens and Wilcken, 1973b). Nevertheless, these findings do not constitute an absolute negation of the concept that adenosine plays a role in the hyperaemic response. Firstly, it is possible that adenosine concentrations produced during the hypoxic or ischaemic period exceed those which can be blocked by aminophylline, since its adenosine-antagonistic action is suggested to be of a competitive type (Scholtholt et al., 1972; Bünger et al., 1975). Secondly, coronary reactive hyperaemia is a complex response, which is not determined solely by locally released adenosine; experimental evidence also supports the participation of local H-ion changes (Raberger et al., 1975), vascular smooth muscle oxygen deprivation (Gellai et al., 1973) and loss of myogenic tone during the period of coronary occlusion (Bayliss, 1902; Eikens and Wilcken, 1974).

On the basis of these considerations we studied the effects of aminophylline on the reactive hyperaemic responses following coronary artery occlusion for 4, 10, and 25 heart beats in the anaesthetised open-chest dog. Aminophylline was infused intracoronarily in order to achieve constant and precise concentrations in the coronary blood and to avoid primary effects of the drug on the peripheral vessels. The chosen dosage was the highest possible which did not affect resting coronary flow and cardiac performance, thus ensuring an unaltered myocardial oxygen consumption. Aminophylline at this dosage is expected to lead to a significant reduction in the vasodilator effect of intracoronarily-administered adenosine, but has not so far been tested on coronary reactive hyperaemia following relatively brief periods of coronary artery occlusion.
Methods

Experiments were carried out on mongrel dogs of either sex between 18 and 24 kg in weight. After premedication with morphine (2 mg·kg⁻¹ s.c.) anaesthesia was induced with 80 mg·kg⁻¹ chloralose intravenously. Following intubation the animals were artificially respirated with a room air/O₂ mixture (6/1) using an Enström respirator. The end-tidal CO₂-concentration (Uras 4, Hartmann & Braun) was kept constant at about 4.5 vol. % by adjustment of the respiratory volume. Anaesthesia was maintained by continuous chloralose infusion (20 mg·kg⁻¹·h⁻¹).

The heart was exposed by left thoracotomy at the 5th intercostal space and suspended in a pericardial cradle. The circumflex branch of the left coronary artery was dissected free near its origin in order to perform blood flow measurements and occlusions. For intracoronary administration of drugs, a double-lumen catheter, with the tip bent at 90°, was inserted into the carotid artery and advanced into the left coronary artery under fluoroscopic control. The precise location of the catheter tip was verified by intracoronary injections of small amounts of adenosine. The animals were anticoagulated with heparin (2 mg·kg⁻¹ i.v.).

Coronary blood flow was measured electro-manometrically using Statham flow probes and flow-meter (SP 2202). Blood pressure was recorded electromanometrically in the aortic arch using a Statham transducer and in the left ventricle using a micro-tip transducer (Millar Instruments). The dP/dt was derived from the left ventricular pressure curve (HSE Physio-Differentiator). The heart rate was integrated from the ECG lead II. All parameters were continuously registered on a Beckman dynograph (Type RM). Oxygen consumption by the left ventricle was calculated according to the formula of Bretschneider (1971) and Bretschneider et al. (1972).

Experimental Protocol

Responses to adenosine injected intracoronarily at a dosage of 4 µg·kg⁻¹ via one lumen of the coronary catheter were recorded. After full decline of the adenosine response the coronary artery was occluded for 4, 10, and 25 heart beats successively (corresponding to a duration of about 3.5, 8.5, and 21.5 s, respectively). This series of coronary challenges was then repeated. The coronary artery was occluded for a defined number of heart beats since Olsson (1970) found a significant correlation between the duration of coronary artery occlusion (measured in number of heart beats) and the additional amount of aminophylline formed in the myocardium. In 6 dogs aminophylline was subsequently infused (in normal saline) via the second lumen of the coronary artery catheter at a rate of 0.5 cm³/min. The concentration of adenosine was selected on the basis of the measured resting coronary flow to give a value of 25 µg·cm⁻³·min⁻¹ coronary blood. Since the coronary blood flow was shown in pilot studies to remain constant over an infusion time of 1 h with the exception of the fleeting adenosine-induced or reactive hyperaemic increases, it may be assumed that the aminophylline concentration remains constant over the 40 min infusion period used in this study. 20 min after the commencement of the aminophylline infusion, coronary challenges as described above, were repeated. In 5 dogs which served as controls the same schedule was performed except that normal saline replaced aminophylline as infusion solution.

The coronary dilator effect of adenosine and the reactive hyperaemic response following coronary artery occlusion were assessed by planimetry of the adenosine-induced and hyperaemic flow volume, respectively. Calculation of the percentage repayment of the flow debt incurred during the occlusion were described in detail elsewhere (Raberger et al., 1975). The statistical evaluation was based on the t test for paired data.

Results

Aminophylline Infusion

As can be seen from the Table aminophylline, infused intracoronarily at the relatively high dosage of 25 µg·cm⁻³·min⁻¹ coronary blood (infusion rate 0.5 cm³/min) had no effect on arterial blood pressure, heart rate, left ventricular dP/dt, and resting coronary flow. Hence, the calculated myocardial oxygen consumption remained almost unchanged.

During pre-infusion conditions the coronary dilator effect of intracoronarily-administered adenosine (4 µg·kg⁻¹) amounted to 12.8 ± 1.0 cm³ (mean ± SEM). 20 min after the commencement of the aminophylline infusion the flow volume produced by adenosine was markedly decreased to 2.6 ± 0.5 cm³, i.e. only 20.3% of the pre-infusion value (Table, Fig. 1). Furthermore, the reactive hyperaemic response and the percentage repayment following coronary artery occlusion for 10 and 25 heart beats decreased in each experiment during the aminophylline infusion, but to a much lesser degree; after both occlusion periods repayments were significantly depressed to about 80% of the pre-infusion values. However, the reactive hyperaemic response and the percentage repayment following coronary occlusion for only 4 heart beats remained virtually unchanged during the aminophylline infusion. The original
**Aminophylline and coronary reactive hyperaemia**

Table  Effects of an intracoronary aminophylline or normal saline infusion on haemodynamics, the coronary dilator effect of adenosine (ASN), and coronary reactive hyperaemia

<table>
<thead>
<tr>
<th></th>
<th>Pre-infusion values</th>
<th>Intracoronary aminophylline infusion</th>
<th>P</th>
<th>Pre-infusion values</th>
<th>Intracoronary saline infusion 0.9%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (min⁻¹)</td>
<td>67 ± 6</td>
<td>70 ± 5</td>
<td>NS</td>
<td>78 ± 13</td>
<td>76 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>MAP (kPa)</td>
<td>17.1 ± 0.8</td>
<td>16.9 ± 0.9</td>
<td>NS</td>
<td>17.6 ± 1.6</td>
<td>17.7 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>dP/dtmax (kPa/s)</td>
<td>357.6 ± 15.9</td>
<td>355.2 ± 24.3</td>
<td>NS</td>
<td>329.9 ± 31.9</td>
<td>323.3 ± 34.1</td>
<td>NS</td>
</tr>
<tr>
<td>CBF (cm³/min)</td>
<td>31 ± 6</td>
<td>31 ± 6</td>
<td>NS</td>
<td>32 ± 2</td>
<td>31 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Calculated MVO₂ per 100 g (cm³/min)</td>
<td>3.64 ± 0.24</td>
<td>3.75 ± 0.2</td>
<td>NS</td>
<td>4.39 ± 0.66</td>
<td>4.33 ± 0.72</td>
<td>NS</td>
</tr>
<tr>
<td>ASN (4 µg·kg⁻¹·i·cor.)</td>
<td>12.8 ± 1</td>
<td>2.6 ± 0.5</td>
<td>&lt;.001</td>
<td>17.8 ± 1.4</td>
<td>19.6 ± 6.3</td>
<td>NS</td>
</tr>
<tr>
<td>Flow volume (cm³)</td>
<td>4.1 ± 0.8</td>
<td>3.9 ± 0.7</td>
<td>NS</td>
<td>4.9 ± 0.8</td>
<td>5.5 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>RHR—4 beats (cm³)</td>
<td>237 ± 38</td>
<td>241 ± 36</td>
<td>NS</td>
<td>337 ± 67</td>
<td>383 ± 63</td>
<td>NS</td>
</tr>
<tr>
<td>% repayment</td>
<td>16.3 ± 2.4</td>
<td>12.2 ± 1.9</td>
<td>-0.01</td>
<td>15.5 ± 1.4</td>
<td>16.1 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>RHR—10 beats (cm³)</td>
<td>339 ± 35</td>
<td>271 ± 27</td>
<td>-0.01</td>
<td>435 ± 68</td>
<td>427 ± 110</td>
<td>NS</td>
</tr>
<tr>
<td>% repayment</td>
<td>31.0 ± 4.9</td>
<td>23.6 ± 3.7</td>
<td>-0.01</td>
<td>37.4 ± 9.4</td>
<td>33.2 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>RHR—25 beats (cm³)</td>
<td>297 ± 39</td>
<td>234 ± 29</td>
<td>-0.01</td>
<td>397 ± 90</td>
<td>385 ± 74</td>
<td>NS</td>
</tr>
</tbody>
</table>

HR, heart rate; MAP, mean aortic blood pressure; CBF, coronary blood flow; MVO₂, myocardial oxygen consumption; RHR, reactive hyperaemic response. Each value represents the mean ± SEM.

Conversion: 1 kPa = 7.519 mmHg.

Tracing of a typical experiment is shown in Fig. 2.

**Normal saline infusion**

Since the serial coronary artery occlusions during aminophylline infusion were carried out 20 to 40 min after the pre-infusion occlusions, a purely time-dependent decrease in the reactive hyperaemic response must be excluded. Therefore, in a further group of animals normal saline was infused intracoronarily at a rate of 0.5 cm³/min. As can be seen from the Table the reactive hyperaemic response and the percentage repayment following coronary artery occlusions for 4, 10, and 25 heart beats, performed 20 min after the commencement of the occlusion.

![Fig. 1](image1.png) Percentage repayment following a 4, 10, and 25 heart beats coronary artery occlusion and the coronary flow volume produced by adenosine (ASN) in the pre-infusion state (open columns) and during an intracoronary aminophylline infusion (hatched columns). Each column represents the mean ± SEM. Significance symbols: **0.01 > P > 0.001, ***P < 0.001.

![Fig. 2](image2.png) Original tracing of the coronary dilator effect of adenosine (ASN) and of coronary reactive hyperaemic response following increasing occlusion periods in the pre-infusion state (control) and during an intracoronary aminophylline infusion.
saline infusion remained virtually unaltered as compared with the pre-infusion values. Furthermore, no significant changes occurred in blood pressure, cardiac performance, coronary blood flow, or in the coronary dilator effect of adenosine during the saline infusion.

Discussion

The coronary dilator effect of adenosine was markedly reduced under the influence of aminophylline in the present study, as has been shown by other workers (Afonso, 1970; Juhran and Dietmann, 1970; Schaumann et al., 1970; Bittar and Pauly, 1971; Curnish et al., 1972; Scholtholt et al., 1972; Eikens and Wilcken, 1973a; Eikens and Wilcken, 1973b; Bünger et al., 1975). In studying the effect of aminophylline on coronary reactive hyperaemia, which is postulated to be mediated by adenosine (Rubio et al., 1969), care was taken to avoid cardiac stimulatory effects of aminophylline (de Gubareff and Sleator, 1965; Marcus et al., 1972), since the rate of endogenous adenosine formation was shown by Rubio and Berne (1969) and Olsson (1970) to be closely related to the myocardial oxygen demands. Myocardial oxygen consumption was kept constant in the present study by employment of the intracoronary route in the administration of aminophylline. Thus, in spite of the high dosage infused, adjusted to an aminophylline concentration of 25 μg·cm⁻³ coronary blood, the left ventricular dP/dt MAX, heart rate, and, hence, myocardial oxygen consumption, calculated according to the formula of Bretschneider (1971) and Bretschneider et al. (1972), did not alter during the whole observation period.

Coronary reactive hyperaemic responses following coronary artery occlusion for 10 and 25 heart beats and the flow volume produced by a single intracoronary injection of adenosine were significantly diminished during the intracoronary aminophylline infusion, whereas the hyperaemic response following a brief occlusion period, ie for 4 heart beats, remained almost unaltered. These findings confirm results obtained previously in this laboratory under similar experimental conditions: both the coronary dilator effect of intracoronarily-administered adenosine and the reactive hyperaemic response following occlusion periods for 10 heart beats and longer were shown to react in a similar direction, ie enhanced during hypercapnic acidosis (Raberger et al., 1975) and depressed under the influence of dihydroergotamine (Raberger et al., 1976), but after brief occlusion periods the reactive hyperaemic response was affected in a reverse manner to the dilator effect of adenosine in both studies. Hence, the present experiments provide further support for the hypothesis that adenosine is a determinant of the reactive hyperaemic flow only after sufficiently long occlusion periods.

However, under the influence of aminophylline the flow volume produced by adenosine was depressed to an extent approximately four times greater than the reactive hyperaemic volume following ischaemic periods for 10 and 25 heart beats; it might, thus, be concluded that endogenously-released adenosine may be only partly involved in these post-occlusion hyperaemias. On the other hand, it cannot be believed per se that the effects of exogenously-administered adenosine should be quantitatively similar to the biological effects of this metabolite produced in situ.

No effects of aminophylline were observed by other authors (Bittar and Pauly, 1971; Juhran et al., 1971; Eikens and Wilcken, 1973a; Eikens and Wilcken, 1973b) on the reactive hyperaemic response following occlusion periods for 4 to 60 s. However, the route of administration was by intracoronary infusion in only one of these studies (Eikens and Wilcken, 1973b) and aminophylline was given at a dosage of approximately 5 μg·cm⁻³ coronary blood, ie amounting to only a fifth of that used in the present experiments. Definite conclusion with regard to the controversial effects of aminophylline on coronary reactive hyperaemia must await more information about the mechanism of adenosine-induced coronary vasodilatation and the exact mode of action of the methylxanthines in antagonising this effect.

The skilful technical assistance of Miss A. Steiner and the assistance of Dr. L. Kastner in preparing the manuscript are gratefully acknowledged.

References