Inhibition of Coronary Vasodilating Action of Dipyridamole and Adenosine by Aminophylline in the Dog

By Skoda Afonso, M.D., Ph.D.

ABSTRACT
Experiments were performed to determine (1) whether intravenously administered aminophylline inhibits the coronary vasodilating effects of intravenous or intracoronary administration of dipyridamole or adenosine and (2) whether aminophylline locally administered in the coronary artery inhibits the vasodilating action of adenosine given intravenously or injected into the coronary artery. Coronary vasodilator responses to dipyridamole or adenosine were determined before and after administration of aminophylline. Intravenous aminophylline was found to inhibit coronary vasodilatation induced by intravenous or intracoronary dipyridamole or adenosine. After intravenous aminophylline, adenosine administered intravenously or into the coronary artery was 2.5 to 4 times less effective in inducing coronary vasodilatation. Aminophylline injected locally into the coronary artery was also effective in inhibiting coronary vasodilatation induced by intravenous and intracoronary adenosine. The mechanism of this inhibitory phenomenon has not been elucidated.

ADDITIONAL KEY WORDS thermodilution flowmeter nitroglycerin acetylcholine coronary sinus blood flow

During our studies to evaluate the coronary vasodilating effects of various agents, we observed that normally effective intravenous doses of dipyridamole, a coronary vasodilator, were not effective in increasing coronary sinus blood flow in dogs previously given aminophylline. In the present paper we report the results of experiments performed to demonstrate inhibition of the coronary vasodilating action of dipyridamole or adenosine by aminophylline. Previous studies had shown that dipyridamole enhances coronary vasodilation induced by intravenously administered adenosine (1, 2). It was further found that dipyridamole decreases the permeability of the red blood cell membrane to adenosine (3, 4), thus preventing entry of adenosine into the red blood cell where it would be inactivated by the intracellular adenosine deaminase. On the basis of these findings, dipyridamole-induced vasodilation could be related to an adenosine action. It was for this reason that the present studies were extended to adenosine also.

The role of catecholamines, if any, in the inhibition of adenosine-induced coronary vasodilation by aminophylline was also investigated after beta-receptor blockade.

Methods
Twenty-six healthy mongrel dogs were used; all were anesthetized with morphine sulfate, 3 mg/kg subcutaneously, followed in 1 hour by intravenous administration of allobarital, 12.5 mg/kg, urethane, 50 mg/kg; monoethylurea, 50 mg/kg; and sodium pentobarbital, 8 mg/kg. Femoral arterial blood pressure was recorded on a direct-writing Sanborn Polyviso, from a Statham strain gauge connected to a percutaneously inserted Courand needle. Coronary sinus blood flow was measured by a thermodilution catheter flowmeter (5) inserted through the jugular vein.

From the Cardiovascular Research Laboratory, University of Wisconsin Medical School, 420 North Charter Street, Madison, Wisconsin 53706.
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Dipyridamole, 2,6-bis (diethanolamino)-4,8-dipiperidino-pyrimido-(5,4-d) pyrimidine, as the intravenous form was supplied as Persantin through the courtesy of Geigy Pharmaceuticals, Ardsley, New York.
and placed under fluoroscopic control in the coronary sinus; flow was recorded on the direct-writing Sanborn Polyviso. Drugs were injected into the right atrium through a cardiac catheter; they were injected into the coronary artery through a no. 5 catheter with a wire cage (2.5 mm diam) affixed at the tip and wedged in its circumflex branch to maintain patency of the vessel and keep the site of injection constant. In dogs 17 through 21, cardiac output was calculated from dye dilution curves obtained with a Gilson dye tracer after injection of indocyanine green into the pulmonary artery. To determine dp/dt, a cardiac catheter was placed in the left ventricle and connected to a Statham strain gauge. Electrical output of the pressure channel was fed into an electronic differentiator and dp/dt recorded on the Sanborn Polyviso.

Heart homogenates were prepared from 10 g of left ventricular myocardium in 90 ml of saline in a Polytron homogenizer. For the assay of adenosine, blood or homogenate mixtures were deproteinized in perchloric acid; filtrates were neutralized to pH 7.0, and their adenosine content was determined by Kalckar's method (6) in a Beckman spectrophotometer.

All dogs were heparinized with 300 units of heparin/kg given intravenously at the beginning of the experiments.

Results

Inhibition of Dipyridamole Coronary Vasodilatation by Aminophylline

In dogs 1 and 2, coronary vasodilator responses to dipyridamole injected into the right atrium were determined before and 15 to 20 minutes after administration of aminophylline into the right atrium. These responses are illustrated in Figure 1. Before aminophylline, 5 mg of dipyridamole produced marked increases in flow which lasted for 15 to 20 minutes in both animals. One hundred mg of aminophylline produced a sharp, brief increase in coronary flow, which returned to control level in 10 minutes. After aminophylline, responses to 5 mg of dipyridamole were almost abolished. Responses to 10 mg of dipyridamole were smaller and briefer than those produced by 5 mg of dipyridamole prior to aminophylline.
Aminophylline, dipyridamole and adenosine

**Figure 2**

CF = coronary sinus blood flow; BP = femoral arterial blood pressure. I: (A) control; (B) responses to 2 mg dipyridamole; (C) 5 minutes after administration of dipyridamole; (D) At 10 minutes. II: (A) after administration of 5 mg dipyridamole. When flow became stable at a higher level, 50 mg aminophylline was given (arrow). Note decrease in flow. (B) At 3 minutes. In the middle of panel blood temperature was verified by disconnecting current to heating coil of flowmeter.

The effect of intravenously administered aminophylline on the coronary vasodilatation produced by intracoronary administration of dipyridamole was studied in dogs 4 and 5. Coronary flow was recorded continuously before and during a constant-rate infusion of 100 μg/min dipyridamole into the coronary artery in dog 4 and 250 μg/min in dog 5. While dipyridamole was being infused and when coronary flow had stabilized at a higher level than the control one, 50 mg of aminophylline was injected into the right atrium. Administration of aminophylline produced in both dogs a transient increase in flow followed by a rapid decrease (Figs. 1 and 3) to the control level prior to dipyridamole infusion. In all dogs of this series, aminophylline antagonized the coronary vasodilating action of dipyridamole.

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to aminophylline. Dog 3 was studied as follows: A control coronary vasodilator response to 2 mg of dipyridamole injected into the right atrium was obtained (Figs. 1 and 2). After coronary flow had returned to control level, 5 mg of dipyridamole was injected into the right atrium. As soon as coronary flow had stabilized at a higher level than that produced by 2 mg of dipyridamole, 50 mg of aminophylline was injected into the right atrium. Aminophylline produced a transient increase in coronary flow followed by a rapid decrease, and flow stabilized at a lower level, close to the control one. Had aminophylline not been given, the coronary vasodilator response to 5 mg of dipyridamole would have been greater and longer lasting than the control one obtained with 2 mg.
CF = coronary sinus blood flow; BP = femoral arterial blood pressure. A: first part of recording is control flow and pressure. At arrow 100 μg/min intracoronary infusion of dipyridamole was begun; note increase of flow. B: continuation of recording A. At arrow 50 mg aminophylline was injected into right atrium. Note decrease in flow. Blood temperature was verified in middle of A and at end of B.

INHIBITION OF ADENOSINE CORONARY VASODILATATION BY AMINOPHYLLINE

Four dogs (6-9) were studied. In the first part of the study, coronary vasodilator responses to constant-rate infusions of adenosine into the right atrium in doses which produced reasonable increases in coronary flow were determined. These infusions were then repeated to determine reproducibility of the responses, and then 150 mg of aminophylline was injected through the right atrial catheter. Ten to 20 minutes after administration of aminophylline and when coronary flow had returned to control level, we again measured the coronary vasodilator responses to infusions of adenosine in doses required to produce increases in coronary flow similar to those obtained in the first part of the study. One hour after administration of aminophylline, responses to the same doses of adenosine were repeated to secure information about the duration of the inhibitory action of aminophylline. Decrease in sensitivity to adenosine was calculated as the ratio between doses of adenosine which produced similar increases in flow after and before aminophylline. Results (Table 1) show that in all dogs, for similar increases in coronary flow, doses of adenosine used after aminophylline were more than twice as great as those used prior to aminophylline. One hour after aminophylline, coronary vasodilator responses to adenosine were still inhibited.

Dogs 10 and 11 were used to demonstrate the inhibition by intravenously administered aminophylline of the coronary vasodilatation induced by intracoronary administration of adenosine. In the first part of the study, coronary vasodilator responses to constant-
Coronary sinus flow (ml/min) during infusions of adenosine before and after aminophylline.

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Wt (kg)</th>
<th>Control Adenosine/min</th>
<th>Before aminophylline</th>
<th>After aminophylline*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intravenous Aminophylline (150 mg)</td>
<td>Intravenous Adenosine (3 mg)</td>
<td>Intravenous Adenosine (3 mg)</td>
</tr>
<tr>
<td>6</td>
<td>19.1</td>
<td>27 33 97</td>
<td>28 33 111</td>
<td>4 times</td>
</tr>
<tr>
<td>7</td>
<td>20.0</td>
<td>28 46 101</td>
<td>28 30 83</td>
<td>2-4 times</td>
</tr>
<tr>
<td>8</td>
<td>21.3</td>
<td>39 71 111</td>
<td>42 57 111</td>
<td>4 times</td>
</tr>
<tr>
<td>9</td>
<td>17.7</td>
<td>23 27 50</td>
<td>23 26 125</td>
<td>2-5 times</td>
</tr>
<tr>
<td>10</td>
<td>24.0</td>
<td>40 68 87</td>
<td>48 52 68</td>
<td>4 times</td>
</tr>
<tr>
<td>11</td>
<td>22.7</td>
<td>46 77 111</td>
<td>50 56 67</td>
<td>4 times</td>
</tr>
<tr>
<td>12</td>
<td>19.1</td>
<td>42 57 90</td>
<td>44 48 53</td>
<td>2.5 times</td>
</tr>
<tr>
<td>13</td>
<td>26.3</td>
<td>40 59 77</td>
<td>39 44 59</td>
<td>2.5 times</td>
</tr>
<tr>
<td>14</td>
<td>20.4</td>
<td>35 44 83</td>
<td>30 36 77</td>
<td>2 times</td>
</tr>
<tr>
<td>15</td>
<td>30.8</td>
<td>47 125</td>
<td>40</td>
<td>2 times</td>
</tr>
<tr>
<td>16</td>
<td>19.5</td>
<td>50 250</td>
<td>48 50</td>
<td>2 times</td>
</tr>
</tbody>
</table>

*Second measurements were 1 hour after aminophylline.

Data in parentheses are doses of adenosine per minute.

Intracoronary infusions of adenosine were determined before and after intracoronary injection of 12 mg of aminophylline in dog 12 and 5 mg in dog 13. Results shown in Table 1 indicate that aminophylline injected into the coronary artery produced a decrease in response to intracoronary adenosine of about 2.5 times in both dogs.

One dog (14) was used to determine the effect of intracoronary aminophylline on coronary vasodilator responses to right atrial infusions of adenosine. After administration of 3.5 mg of aminophylline there was a decrease in sensitivity to intravenous adenosine of 2 times.

Dog 15 and 16 were treated with 2 mg/kg of propranolol intravenously. Coronary vasodilator responses to a constant-rate infusion of adenosine into the right atrium were determined before and after intravenous injection of 230 mg of aminophylline in dog 15 and 150 mg in dog 16. Results (Table 1) indicate that...
after blockade of beta receptors aminophylline was still capable of inhibiting coronary vasodilator responses to adenosine. Doses of adenosine which produced marked increases in flow before aminophylline were not effective in increasing flow after aminophylline.

Aminophylline and adenosine each produce changes in cardiac work, heart rate, and myocardial contractility that might influence coronary blood flow. To determine whether these actions could be responsible for the observed inhibition of adenosine-induced coronary vasodilation by aminophylline, five dogs (17-21) were studied in the following way. Control measurements of cardiac output, femoral arterial blood pressure, heart rate, dp/dt, and coronary sinus blood flow were obtained. Then adenosine was infused in the right atrium at a constant rate in a dose that produced a marked increase in coronary blood flow. After flow had stabilized at a high level, cardiac output, blood pressure, heart rate, dp/dt, and coronary flow were measured again. While adenosine was still being infused, 150 mg of aminophylline was administered into the right atrium. Aminophylline produced a transient increase in coronary flow and then flow started to decrease. Two to 3 minutes after aminophylline administration and while adenosine was still being infused, the same variables were again measured. This timing was chosen to have a cardiac output comparable to the one prior to aminophylline and a higher heart rate and dp/dt. Results are
AMINOPHYLLINE, DIPYRIDAMOLE AND ADENOSINE

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CONTROL 1004 mg/min. L.C.

FIGURE 5

CF = coronary sinus blood flow; BP = femoral arterial blood pressure. I: Adenosine responses before aminophylline: in the middle of the recording, flow and pressure during 4μg/min intracoronary adenosine; at end, flow and pressure during 8 μg/min adenosine. II: Adenosine responses after 12 mg intracoronary aminophylline. (A) control; (B) at beginning 4 μg/min intracoronary adenosine and at the end during 8 μg/min infusion; (C) during 20 μg/min infusion. Blood temperature was verified twice in I and II: B and C.

given in Table 2. Before aminophylline and during the infusions of adenosine for an average left ventricular work of 6.0 kg-m/min, a heart rate of 163 beats/min and dp/dt of 3160 mm Hg/sec, average coronary flow was 146 ml/min. After aminophylline and during the same variables were again measured. This decreased to 67 ml/min even though dp/dt, heart rate, and cardiac work increased.

ACTION OF AMINOPHYLLINE ON CORONARY VASODILATION INDUCED BY NITROGLYCERIN AND ACETYLCHOLINE

Five dogs (22-26) were used to investigate whether the inhibitory action of aminophylline extends to other types of coronary vasodilators. Coronary vasodilator responses to intravenous injections of nitroglycerin and of acetylcholine were first determined. Each agent was used in two doses, one chosen to produce a small increase in coronary flow and a second that was twice as great. Then responses to the same doses used previously were redetermined after intravenous administration of 150 mg of aminophylline. The recorded responses were integrated and calculated in terms of volume of blood (ml). Values shown in Table 3 indicate that after aminophylline the responses are comparable to the respective ones obtained before aminophylline. Aminophylline did not inhibit coronary vasodilatation induced by nitroglycerin or acetylcholine.

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To investigate the biochemical basis of inhibition of adenosine action the following experiments were performed.

A. The time course of disappearance of adenosine added to dog blood was determined in the presence and in the absence of aminophylline. Blood mixtures containing (1) 20 µg/ml adenosine and (2) 20 µg/ml of adenosine and 150 µg/ml aminophylline were incubated in a Dubnoff shaker at 37°C. Aliquots of the mixtures were taken immediately after addition of adenosine and at known time intervals, and their adenosine content was determined. Seven determinations were performed. Rates of disappearance of adenosine calculated on the basis of 50% inactivation are given in Table 4. Adenosine in blood disappeared at an average rate of 0.74 µg/min/ml blood in the absence of aminophylline and at a rate of 0.70 µg/min/ml blood in the presence of aminophylline. Aminophylline had no influence on the rate of disappearance of adenosine added to blood.

B. To determine whether aminophylline influences the rate of destruction of adenosine added to heart homogenates, homogenates were prepared containing: (1) 100 µg/ml adenosine and (2) 100 µg/ml adenosine and 300 µg/ml aminophylline. These were incubated at 37°C. The adenosine content of aliquots taken at known time intervals of incubation was determined. No accelerating effect by aminophylline on the rate of destruction of adenosine in heart homogenates could be demonstrated. In the absence and in the presence of aminophylline, average rates of adenosine destruction were 151 and 148 µg/min/g heart muscle, respectively.

C. The time course of inactivation of adenosine by adenosine deaminase was followed in phosphate-buffered (pH 7.5), incubated (37°C) solutions containing: (1) 20 µg/ml adenosine and 0.05 µg/ml adenosine deaminase and (2) 20 µg/ml adenosine, 0.05 µg/ml adenosine deaminase and 150 µg/ml aminophylline. Three experiments were performed.

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Adenosine-Deaminase, C. F. Boehringer & Soehne, Mannheim.

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TABLE 3

Coronary Blood Flow (ml/min) after Nitroglycerin and Acetylcholine before and after Aminophylline

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Wt (kg)</th>
<th>Before aminophylline</th>
<th>After aminophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First dose</td>
<td>Double dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>18.1</td>
<td>5.0</td>
<td>9.0</td>
</tr>
<tr>
<td>23</td>
<td>23.6</td>
<td>9.0</td>
<td>26.0</td>
</tr>
<tr>
<td>24</td>
<td>20.0</td>
<td>12.3</td>
<td>19.9</td>
</tr>
<tr>
<td>25</td>
<td>24.0</td>
<td>8.5</td>
<td>13.8</td>
</tr>
<tr>
<td>26</td>
<td>20.5</td>
<td>11.5</td>
<td>17.9</td>
</tr>
<tr>
<td>23</td>
<td>23.6</td>
<td>1.9</td>
<td>3.3</td>
</tr>
</tbody>
</table>

First doses of nitroglycerin in dogs 22, 23, and 24 were 50, 100, and 20 μg, respectively. First doses of acetylcholine in dogs 25, 26, and 23 were 10, 5, and 2 μg, respectively.

TABLE 4

Rate of Disappearance of Adenosine in Blood (μg/min/ml blood)

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without aminophylline</td>
<td>0.40</td>
<td>0.57</td>
<td>1.01</td>
<td>1.00</td>
<td>1.01</td>
<td>0.51</td>
<td>0.65</td>
<td>0.74</td>
</tr>
<tr>
<td>With aminophylline</td>
<td>0.43</td>
<td>0.48</td>
<td>1.18</td>
<td>0.77</td>
<td>1.00</td>
<td>0.43</td>
<td>0.59</td>
<td>0.70</td>
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</table>

Rate of Inactivation of Adenosine in Heart Homogenates (μg/min/g heart)

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<tr>
<th>Experiment number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without aminophylline</td>
<td>77</td>
<td>143</td>
<td>209</td>
<td>185</td>
<td>151</td>
</tr>
<tr>
<td>With aminophylline</td>
<td>91</td>
<td>143</td>
<td>200</td>
<td>156</td>
<td>148</td>
</tr>
</tbody>
</table>

Rate of Inactivation of Adenosine by Adenosine Deaminase (μg/min)

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without aminophylline</td>
<td>2.6</td>
<td>2.6</td>
<td>1.4</td>
<td>2.2</td>
</tr>
<tr>
<td>With aminophylline</td>
<td>2.5</td>
<td>2.6</td>
<td>1.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Aminophylline had no influence on the rate of deamination of adenosine by adenosine deaminase.

Discussion

Experiments performed in this study consisted essentially of comparing coronary vasodilator effects of dipyridamole or adenosine before and after aminophylline. In all instances after aminophylline, both dipyridamole and adenosine were less effective in causing coronary vasodilatation. Results in dogs used to investigate the role of cardiac work, myocardial contractility and heart rate indicate that the decreased coronary vasodilator responses to adenosine cannot be ascribed to the changes in these factors. Aminophylline administered into the right atrium or locally into the coronary artery was effective in inhibiting coronary vasodilatation induced by intravenous or intracoronary administration of adenosine, and the degree of inhibition was the same for all these combinations.

We also found that aminophylline does not influence coronary vasodilatation induced by nitroglycerin and acetylcholine and thus it seems that the interaction between aminophylline and adenosine or dipyridamole is a selective one.

We believe the results establish satisfactorily that an inhibitory phenomenon occurs. However, its nature is not yet known. Exogenous adenosine injected in blood is inactivated by adenosine deaminase which is widespread in tissue and blood. The inhibition could be related to a blood-borne phenomenon with aminophylline acting by accelerating the rate of adenosine disappearance. However, this possibility can be ruled out in view of the finding that aminophylline had no
influence on the rate of disappearance of adenosine in whole blood. The inhibition could be due to an accelerated rate of adenosine inactivation by the lung, but this is not the case because aminophylline produced the same degree of inhibition on coronary vasodilatation induced by right atrial and intracoronary administration of adenosine. The finding that aminophylline injected into the coronary artery is also effective in inhibiting vasodilator effects of intracoronary adenosine suggests that aminophylline could be either acting on the myocardium by accelerating the rate of adenosine inactivation there or on vascular smooth muscle in the coronary circulation. No accelerating action by aminophylline on the rate of adenosine inactivation could be demonstrated in heart homogenates. These negative results do not support the first suggestion, but the possibility that aminophylline may have such an action in the intact myocardium has not been ruled out in this study. We do not yet know what is the mechanism of this inhibitory phenomenon. There is little information on inhibition of adenosine actions by any other agent which could be of help in elucidating the currently reported phenomenon. Phenoxybenzamine inhibits coronary vasodilatation induced by adenosine in isolated guinea pig and rat heart (7); however, in dogs we found that it does not inhibit coronary vasodilator responses to adenosine (unpublished observations). Caffeine was found to antagonize the depres-
sant effects of adenosine on contraction and action potentials of guinea pig and human atrial muscle (8). On the basis of this observation, a more likely suggestion is that aminophylline acts on the smooth muscle of the coronary vascular bed to antagonize the action of adenosine at this site.

References
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SKODA AFONSO

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