Inhibition of Sympathetic Neurotransmission in Canine Blood Vessels by Adenosine and Adenine Nucleotides

RAYMOND H. VERHAEGHE, PAUL M. VANHOUTTE, AND JOHN T. SHEPHERD

SUMMARY  Adenosine and the adenine nucleotides caused a greater relaxation of strips of canine saphenous vein and tibial artery when they had been contracted by nerve stimulation than by exogenous norepinephrine. An infusion of adenosine into the dogs' lateral saphenous vein, perfused at constant flow, caused a greater relaxation of this vein when constricted by electrical stimulation of the lumbar sympathetic chain than by exogenous norepinephrine. That this difference was due to inhibition by these compounds of the output of neurotransmitter from the sympathetic nerve endings was demonstrated by column chromatographic analysis of the radioactivity in the superfusion fluid of vein strips, previously incubated with tritiated norepinephrine. Both adenosine and adenosine triphosphate (10⁻⁵ M) reduced the efflux of ³H-norepinephrine during nerve stimulation with electrical impulses. Adenosine also reduced the efflux caused by potassium (30 mM), but not that caused by tyramine (6 x 10⁻⁶ M). Theophylline antagonized the inhibitory effect of adenosine on the sympathetic neurotransmission. We found that at 4 x 10⁻⁴ M adenosine triphosphate still caused a decreased efflux of neurotransmitter during electrical stimulation, but with adenosine the ³H-norepinephrine efflux no longer decreased and the overflow of deaminated compounds increased. Furthermore, the same concentration of adenosine increased the efflux of ³H-norepinephrine and deaminated compounds in unstimulated strips, and the increase of ³H-norepinephrine was enhanced after monoamine oxidase inhibition. Thus, we conclude that at higher concentrations adenosine increases the intraneuronal leakage of norepinephrine out of the storage vesicles.

ADENOSINE and the adenine nucleotides by a local action on blood vessels cause their dilatation. This has been demonstrated both in vivo and in vitro.¹⁻⁴ There is evidence that these substances can inhibit neuromuscular transmission in the rat phrenic nerve-diaphragm and frog sartorius preparations⁵ and cholinergic neurotransmission in the intestinal wall.⁶ The present experiments were performed to determine whether adenosine and related nucleotides inhibit adrenergic neurotransmission in canine blood vessels.

Methods

STUDIES IN VITRO

Helical strips of lateral saphenous veins and anterior tibial arteries, isolated from dogs (15-25 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv), were placed in an organ bath filled with Krebs-Ringer solution (NaCl, 118.2 mM; KCl, 4.7 mM; MgSO₄, 1.2 mM; KH₂PO₄, 1.2 mM; CaCl₂, 2.5 mM; NaHCO₃, 25 mM; calcium disodium ethylenediaminetetraacetate (EDTA) 0.026 mM; and glucose, 5.5 mM) maintained at 37°C and aerated with a 95% O₂-5% CO₂ mixture. The strips were attached to a force transducer (Grass FT 03C) for isometric tension recording. The transducer was mounted on a movable support allowing fine adjustments of the strip length and connected to a pen writing recorder (Gould Brush 220). For electrical stimulation of the preparations, two rectangular platinum electrodes (20- by 4-mm surface, 0.5 mm thick) were placed parallel to vein strips. The electrical impulses were of rectangular waves (0.2-20 Hz, 9 V, 2 msec) from a direct current power supply and switching transistor (RCA 2N-3034) triggered by a stimulator (Grass SM6C).

An equilibration period (20-40 minutes) the length of each strip was increased by 2-mm increments until the contraction caused by a standard electrical stimulus (10 Hz for 10 seconds) reached a maximum.⁷ The length at which this occurred was maintained throughout the experiment.

The following pharmacological agents were used: adenosine (Aldrich), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) (Sigma), l-norepinephrine bitartrate (Winthrop), tyramine hydrochloride (Abbott), pargyline hydrochloride (Abbott), and theophylline (Sigma). All doses are expressed as final bath concentrations. The drugs were removed from the bath solution by overflowing the preparation with aerated Krebs-Ringer solution at 37°C.

When the effect of increasing concentrations of adenosine or nucleotides was investigated, sustained contractions were obtained with either electrical stimulation (2 Hz) or norepinephrine (6 x 10⁻⁷ M). After the tension had become maximal (control value), the selected concentration of adenosine or one of the nucleotides was added to the bath. Usually 4 minutes was sufficient for the tension to stabilize at its new level and this value was taken as the response to adenosine or the nucleotide. Only one agent was tested on each vascular strip. All strips were allowed to recover for 10 minutes between two consecutive contractions.

From the Department of Physiology and Biophysics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota, and Department of Internal Medicine, Universitaire Instelling, Antwerpen, Antwerp, Belgium.

Supported in part by Research Grant HL-5883 from the National Institutes of Health, Public Health Service.

Dr. Verhaeghe is a recipient of a Public Health Service International Research Fellowship (F05-TW-2167) on leave of absence from the University of Leuven, Belgium.

Address for reprints: Dr. John T. Shepherd, Mayo Clinic and Mayo Foundation, 200 First Street, S.W., Rochester, Minnesota 55901.

Received June 9, 1976; accepted for publication September 8, 1976.
Cumulative frequency-response curves to electrical stimulation or dose-response curves to norepinephrine were obtained first in control solution and repeated after incubation for 10 minutes in solution containing adenosine or one of the nucleotides. Repeated washings were made and a second control curve was obtained after the experimental curve. The mean effective dose (ED_50) and maximal response of the second control curve always differed by less than 5% from those of the first. Both control curves were averaged for plotting the results and for statistical calculation. Dose ratios were calculated as the displacement of the curve at 50% of the maximal response.

In some experiments, helical strips of saphenous veins were incubated for 4 hours in Krebs-Ringer solution containing 1.25 x 10^{-7} M 7-3H-norepinephrine (specific activity, 9.8 Ci/mmol, New England Nuclear). At the end of the incubation, the strips were rinsed in fresh Krebs-Ringer solution and mounted for superfusion. Each preparation was suspended in a moist tunnel-shaped chamber maintained at 37°C and superfused at 3 ml/min by a constant flow roller pump (Holter, model 911) with aerated (95% O_2 and 5% CO_2) Krebs-Ringer solution prewarmed to perfuse at 37°C. The strip was connected to a force transducer (Grass FT 03C) for isometric tension recording (Clevite Brush Mark 250). Two platinum wires (10 cm long, 0.5 mm in diameter) were fixed parallel to the strip. Both vein strip and electrodes were perfused continuously. Electrical stimulation was similar to that in the organ bath experiments. The original tension was set at 2 g, and sampling at 2-minute intervals was started after an equilibration period of 20 minutes. Insta-Gel emulsifier (10 ml) (Packard) was added to 1-ml samples before measurement of total radioactivity in a liquid scintillation counter. Corrections for quenching were made with an Instagel standard in each counting vial. The data, Student's t-test was used.

The number of strips reported for each group of experiments is also the number of dogs used. All data are expressed as mean ± se. For statistical analysis of the data, Student's t-test was used.

RESULTS

Effect of Adenosine, ADP, and ATP on Response of Vascular Strips to Electrical Stimulation and Norepinephrine

The original record of an experiment in which a single concentration of adenosine (10^{-4} M) was added during a sustained contraction of a saphenous vein strip caused by electrical stimulation (2 Hz) is shown in Figure 1. Adenosine induced an immediate relaxation which was maximal within the 1st minute and was followed by a slight increase in tension. The relaxation was rapidly and completely reversible upon removal of adenosine. In identical experiments on six strips the depression of the response to electrical stimulation (mean increase in tension, 1.30 ± 0.13 g) averaged 54.6 ± 4.1% after 1 minute and 44.9 ± 4.1% after 6 minutes. The tension returned to 100.7 ± 3.1% of the control level after washing out the nucleoside. The same concentration of adenosine had no effect on the basal tension in these strips.

In some samples, norepinephrine was separated from its metabolites in the superfusate by column chromatography. In a first step, catechol compounds were separated from noncatechol metabolites and from tritiated water by adsorption on alumina at pH 8.4 and subsequent elution with acid. In a second step, norepinephrine was separated from deaminated compounds in the alumina eluate and normetanephrine from deaminated O-methylated metabolites in the alumina effluent by adsorption on a Dowex 50-X4 resin and subsequent elution with acid. The procedure is described in detail elsewhere. In a few experiments the deaminated compounds were further split by a two-step elution from the alumina: 3,4-dihydroxyphenylglycol (DOPEG) and norepinephrine were first eluted with acetic acid (0.2 N) and thereafter 3,4-dihydroxyphenylacetic acid (DOMA) with hydrochloric acid (0.2 N and 1 N in succession). Norepinephrine was subsequently separated from DOPEG on Dowex resin. Radioactivity was measured in a liquid scintillation counter on 1-ml samples of effluents and eluates from the columns.
significantly ($P < 0.01$) larger depression (average, $52.8 \pm 8.3\%$) of the reaction to electrical stimulation.

Similar studies were carried out in three series of six vein strips, using in random sequence several concentrations ($10^{-6}$ to $5 \times 10^{-4} \text{ M}$) of adenosine, ADP, and ATP, respectively. The results are shown in Figure 2. In concentrations lower than $5 \times 10^{-5} \text{ M}$, the three purine compounds depressed only the response to electrical stimulation; the responses to both electrical stimulation and norepinephrine were depressed with higher concentrations, but the relaxation during the reaction to electrical stimulation always remained significantly greater than that during the norepinephrine-induced contraction ($P < 0.01$).

In six other groups of six veins each, either frequency-response curves to electrical stimulation (0.5-15 Hz) or cumulative dose-response curves to norepinephrine ($6 \times 10^{-4}$ to $3 \times 10^{-3} \text{ M}$) were obtained (Fig. 3). Adenosine, ADP, and ATP ($10^{-4} \text{ M}$) significantly depressed the frequency-response curve to electrical impulses; the dose ratios were 1.9, 1.9, and 2.0, respectively. In contrast, the depression of the dose-response curves to norepinephrine by the same concentrations of the three adenine compounds was significant only for the lower doses of the catecholamine; the dose ratios were 1.3, 1.25, and 1.25, respectively.

Six other strips were treated with cocaine ($3 \times 10^{-5} \text{ M}$) for 30 minutes before obtaining a frequency-response curve to electrical stimulation (0.2-20 Hz). Adenosine ($10^{-4} \text{ M}$) depressed this curve to the same extent as in untreated strips, the dose ratio being 2.0.

A pair of helical strips was obtained from each of six
tibial arteries. One member of each pair was contracted by electrical stimulation, the other by norepinephrine. Adenosine (10⁻⁴ M) depressed the contractions caused by each stimulation frequency significantly more than comparable contractions caused by norepinephrine (Table 1).

**EFFECT OF ADENOSINE AND ATP ON OUTPUT OF ³H-NOREPINEPHRINE IN SAPHENOUS VEIN STRIPS**

The basal efflux of total radioactivity, ³H-norepinephrine, and its metabolites was unaltered by adenosine (10⁻⁵ M) in six unstimulated strips previously incubated with labeled neurotransmitter. In contrast, in six other strips, a higher concentration of adenosine (4 x 10⁻⁴ M) resulted in a rise of the downward slope of the radioactivity efflux curve (Fig. 4). This was due mainly to an augmented outflow of deaminated metabolites, DOPEG (23.8 ± 3.2%) and DOMA (20.8 ± 7.1%), together with a slight increase in ³H-norepinephrine (11.3 ± 3.2%). Four additional strips were incubated for 30 minutes with the monoamine oxidase inhibitor pargyline (1.5 x 10⁻⁴ M) before superfusion; under these circumstances there was a significantly (P < 0.01) larger increase in the efflux of intact neurotransmitter (35.5 ± 3.9%) with the same concentration of adenosine (Table 2).

ATP (10⁻⁵ M) caused a poorly sustained contraction of five unstimulated strips as described by Furchgott, but had no effect on the slope of the radioactivity efflux curve or on the efflux of neurotransmitter or its metabolites. ATP (4 x 10⁻⁴ M) caused a larger increase in tension in seven other strips together with a slight depression of the radioactivity efflux curve, because of a decrease in deaminated O-methylated metabolites (19.6 ± 3.9%) and normetanephrine (20.8 ± 4.8%) (Fig. 4 and Table 2).

**EFFECT OF ADENOSINE AND ATP ON OUTPUT OF ³H-NOREPINEPHRINE DURING NERVE STIMULATION IN SAPHENOUS VEIN STRIPS**

In eight strips electrical stimulation (2 Hz) caused an increase in tension together with an augmented efflux of total radioactivity and ³H-norepinephrine into the superfusion fluid. Adenosine (10⁻⁵ M) depressed the contraction by 33.0 ± 4.3%, the overflow of total radioactivity by 45.3 ± 8.1% and in ³H-norepinephrine by 27.9 ± 3.2% (Fig. 7). ATP (4 x 10⁻⁴ M) caused a poorly sustained contraction of the strips, a higher concentration of adenosine (4 x 10⁻⁴ M) resulted in a rise of the downward slope of the radioactivity efflux curve (Fig. 4). This was due mainly to an augmented outflow of deaminated metabolites, DOPEG (23.8 ± 3.2%) and DOMA (20.8 ± 7.1%), together with a slight increase in ³H-norepinephrine (11.3 ± 3.2%). Four additional strips were incubated for 30 minutes with the monoamine oxidase inhibitor pargyline (1.5 x 10⁻⁴ M) before superfusion; under these circumstances there was a significantly (P < 0.01) larger increase in the efflux of intact neurotransmitter (35.5 ± 3.9%) with the same concentration of adenosine (Table 2).

ATP (10⁻⁵ M) caused a poorly sustained contraction of five unstimulated strips as described by Furchgott, but had no effect on the slope of the radioactivity efflux curve or on the efflux of neurotransmitter or its metabolites. ATP (4 x 10⁻⁴ M) caused a larger increase in tension in seven other strips together with a slight depression of the radioactivity efflux curve, because of a decrease in deaminated O-methylated metabolites (19.6 ± 3.9%) and normetanephrine (20.8 ± 4.8%) (Fig. 4 and Table 2).

In 11 other strips, adenosine (4 x 10⁻⁴ M) depressed the contraction obtained with electrical stimulation (2 Hz) by 41.8 ± 4.2%, but not the efflux of total radioactivity. In three strips, the superfusion analysis of the superfusate for six of these strips showed that there was no decrease in the ³H-norepinephrine; there was an increase in deaminated metabolites (20.5 ± 3.7%) and a decrease in normetanephrine (8.2 ± 3%) (Fig. 6).

In eight strips ATP (10⁻⁵ M) depressed the contraction by 17.3 ± 3.2% and the overflow of total radioactivity by 22.9 ± 2.4% during electrical stimulation at 1 Hz; a decrease in ³H-norepinephrine (24.0 ± 4.0%) was found by analysis of the superfusate of five of these strips. In nine other strips, ATP (4 x 10⁻⁴ M) depressed the contraction by 20.9 ± 3.5% after an initial slight increase in tension and the overflow of total radioactivity by 33.5 ± 3.7%. After removal of the nucleotide there was a further transient decrease in tension (12.6 ± 1.2%). In the five strips in which tests were made, ATP depressed the efflux of ³H-norepinephrine (37.7 ± 4.7%), deaminated O-methylated metabolites (13.6 ± 3.9%), and normetanephrine (14.8 ± 4.0%) (Fig. 7).

In five strips, a change in the K⁺ concentration of the superfusion fluid from 5.9 to 30 mM caused an increase in tension, in radioactivity of the superfusate, and in ³H-norepinephrine efflux. Adenosine (10⁻⁵ M) depressed the contraction by 42.8 ± 5.1% and the increase in radioactivity by 45.3 ± 8.1% and in ³H-norepinephrine by 27.9 ± 3.2%.

**Table 1**

<table>
<thead>
<tr>
<th>Electrical stimulation (g)</th>
<th>Norepinephrine (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><strong>Adenosine, 10⁻⁴ M</strong></td>
</tr>
<tr>
<td><strong>Hz</strong></td>
<td><strong>(g)</strong></td>
</tr>
<tr>
<td>0.5</td>
<td>1.75 ± 0.46</td>
</tr>
<tr>
<td>2</td>
<td>3.46 ± 0.73</td>
</tr>
<tr>
<td>10</td>
<td>7.2 ± 1.01</td>
</tr>
<tr>
<td>15</td>
<td>8.21 ± 1.03</td>
</tr>
</tbody>
</table>

**Values shown as mean ± ss for six dogs.**

* Increase in tension caused by stimulus in absence of adenosine.

† Percent decrease of response by adenosine.

§ Response to norepinephrine is significantly less depressed than comparable response to electrical stimulation (P < 0.01 and < 0.001, respectively).
Table 2: Effect of Adenosine and ATP on Basal Efflux of \textsuperscript{3}H-norepinephrine and Metabolites in Unstimulated Saphenous Vein Strips

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Norepinephrine</th>
<th>Deaminated metabolites</th>
<th>Deaminated O-methylated metabolites</th>
<th>Normetanephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.6 ± 1.1</td>
<td>22.7 ± 6.3</td>
<td>23.5 ± 2.3</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Adenosine (10\textsuperscript{-5} M)</td>
<td>3.7 ± 1.0</td>
<td>24.0 ± 6.9</td>
<td>25.5 ± 2.5</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>4.9 ± 0.8</td>
<td>15.8 ± 2.2*</td>
<td>11.7 ± 1.1†</td>
<td>36.3 ± 6.3</td>
</tr>
<tr>
<td>Adenosine (4 × 10\textsuperscript{-4} M)</td>
<td>5.5 ± 1.0‡</td>
<td>19.3 ± 2.3*</td>
<td>14.0 ± 1.0†</td>
<td>37.6 ± 7.3</td>
</tr>
<tr>
<td>Control</td>
<td>7.8 ± 1.7</td>
<td>1.0 ± 0.6</td>
<td>22.1 ± 1.6</td>
<td>14.2 ± 2.6</td>
</tr>
<tr>
<td>Adenosine (4 × 10\textsuperscript{-4} M)</td>
<td>10.5 ± 2.2§</td>
<td>1.4 ± 0.9</td>
<td>21.4 ± 1.9</td>
<td>14.0 ± 3.0</td>
</tr>
<tr>
<td>ATP (10\textsuperscript{-3} M)</td>
<td>2.9 ± 0.4</td>
<td>27.3 ± 2.6</td>
<td>29.7 ± 2.4</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Control</td>
<td>4.8 ± 0.9</td>
<td>25.5 ± 5.0</td>
<td>20.2 ± 3.5</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>ATP (4 × 10\textsuperscript{-4} M)</td>
<td>5.2 ± 1.0</td>
<td>27.5 ± 5.5</td>
<td>16.2 ± 2.2§</td>
<td>2.1 ± 0.3§</td>
</tr>
</tbody>
</table>

All data are expressed as dpm × 10\textsuperscript{3} per minute of superfusion (mean ± se); control = mean of value taken before and after exposure to adenosine or ATP for each strip; n = number of strips.

* P < 0.05 (value significantly different from control).
† P < 0.01 (value significantly different from control).
‡ P < 0.05 (value significantly different from control).
§ P < 0.01 (value significantly different from control).

± 5.6%. In five other strips, adenosine (10\textsuperscript{-5} M) added during contractions induced by tyramine (6 × 10\textsuperscript{-4} M) caused a relaxation (24.5 ± 4.1%) but no change in radioactivity and \textsuperscript{3}H-norepinephrine efflux (Fig. 8).

Five strips were superfused for 20 minutes with Krebs-Ringer solution containing theophylline (10\textsuperscript{-3} M). During subsequent electrical stimulation (1 Hz), adenosine (10\textsuperscript{-3} M) depressed the contraction by 6.4 ± 1.0% and the efflux of radioactivity by 7.7 ± 1.6%; the \textsuperscript{3}H-norepinephrine efflux was slightly but not significantly depressed (4.9 ± 3.8%). Five other strips were used as a control and stimulated at the same frequency. The same concentration of adenosine depressed the contraction by 45.8 ± 7.2%, the efflux of radioactivity by 41.4 ± 9.0%, and the efflux of \textsuperscript{3}H-norepinephrine by 50.0 ± 9.3%. All differences between both groups are significant (P < 0.01) (Fig. 9).

![Figure 5](http://circres.ahajournals.org/)

**Figure 5** Effect of adenosine (10\textsuperscript{-5} M) on tension (top), total radioactivity in the superfusate (middle), and efflux of \textsuperscript{3}H-norepinephrine (NE) and metabolites (bottom) in saphenous vein strips during electrical stimulation. Data are shown as mean ± se. Arrows indicate time at which samples were collected for column chromatography. NMN = normetanephrine.

![Figure 6](http://circres.ahajournals.org/)

**Figure 6** Effect of adenosine (4 × 10\textsuperscript{-4} M) on tension (top), total radioactivity in the superfusate (middle), and efflux of \textsuperscript{3}H-norepinephrine and metabolites (bottom) in saphenous vein strips during electrical stimulation. Data are shown as mean ± se. Arrows indicate time at which samples were collected for column chromatography. NMN = normetanephrine.
IN VIVO EFFECT OF ADENOSINE ON RESPONSE OF SAPHENOUS VEIN TO LUMBAR SYMPATHETIC STIMULATION AND INFUSION OF NOREPINEPHRINE

In seven dogs, the lateral saphenous vein was perfused at constant flow (100 ml/min) with autologous blood. The infusion of adenosine (5 x 10^{-5} M) for 3 minutes did not affect the perfusion pressure in the absence of stimulation. Sustained venoconstrictions were obtained with two frequencies (2 and 10 Hz) of electrical stimulation of the lumbar sympathetic chain; in each dog venoconstrictions of comparable amplitude to those obtained with the two stimulation frequencies also were evoked by selecting appropriate concentrations of norepinephrine (1 ± 0.4 x 10^{-6} M and 4 ± 0.9 x 10^{-6} M, respectively). Adenosine, infused at 5 x 10^{-5} M for 3 minutes during these venoconstrictions, depressed the response to the two frequencies of lumbar sympathetic stimulation significantly more than those caused by the corresponding concentrations of norepinephrine (Table 3).

Discussion

The comparison of the effect of adenosine during contractions of isolated blood vessels caused by electrical stimulation and exogenously applied norepinephrine is a first step to establish whether this nucleoside has an inhibiting effect on the sympathetic nerves. The interpretation of the results of this comparison depends on the evidence that contractions of vascular strips caused by electrical stimulation are due to release of norepinephrine from the adrenergic nerve endings rather than to direct excitation of the smooth muscle cells. The contractions of isolated canine saphenous veins in response to electrical field stimulation as used in the present experiments are abolished by blockade of peripheral adrenergic transmission with bretylium tosylate or tetrodotoxin, by reserpine treatment, by chronic sympathectomy, and by α-receptor blockade. Electrical stimulation augments the efflux of norepinephrine in preparations previously incubated with labeled transmitter. Thus, the contractions of the isolated blood vessel strips caused by electrical stimulation in the present experiments are due to evoked release of catecholamines from the adrenergic nerve terminals.

Whether increasing concentrations of adenosine are tested on the response to a constant stimulus, or vice versa, the contractions induced by nerve stimulation are more depressed than those caused by exogenous norepinephrine. This indicates that in the vein contracted by nerve stimulation, part of the relaxation caused by adenosine is due to interference with sympathetic neurotransmission. This is confirmed by the fact that adenosine in a concentration which has no effect on the basal efflux of
Table 3

Effect of Adenosine on Comparable Venoconstriction Induced by Lumbar Chain Stimulation and Norepinephrine in Canine Saphenous Veins Perfused with Autologous Blood

<table>
<thead>
<tr>
<th>Lumbar chain stimulation</th>
<th>Adenosine, 5 × 10⁻⁵ m²</th>
<th>Norepinephrine</th>
<th>Adenosine, 5 × 10⁻⁵ m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hz</td>
<td>Control*</td>
<td>Adenosine (%)</td>
<td>Control*</td>
</tr>
<tr>
<td></td>
<td>(mm Hg)</td>
<td>(mm Hg)</td>
<td>(mm Hg)</td>
</tr>
<tr>
<td>2</td>
<td>71.5 ± 13.7</td>
<td>61.8 ± 6.0</td>
<td>70.3 ± 16.5</td>
</tr>
<tr>
<td></td>
<td>10.19 ± 5.4</td>
<td>26.6 ± 5.4</td>
<td>10.26 ± 5.3</td>
</tr>
<tr>
<td>10</td>
<td>4 ± 0.9</td>
<td>16.9 ± 6.3§</td>
<td>5.6 ± 5.1§</td>
</tr>
</tbody>
</table>

Values shown as mean ± se for seven dogs.
* Increase in perfusion pressure caused by stimulus in absence of adenosine.
§, H Response to norepinephrine is significantly less depressed than comparable response to lumbar sympathetic stimulation (P < 0.001 and P < 0.02, respectively).

ADP and ATP also inhibit adrenergic neurotransmission. This can be concluded from the experiments which demonstrate that both nucleotides depress the response of saphenous veins to nerve stimulation more than to exogenous norepinephrine. The decrease in the efflux of labeled neurotransmitter by ATP during electrical stimulation supports this conclusion. The response to electrical stimulation is depressed by concentrations of the three compounds which do not affect the response to direct α-adrenergic activation of the smooth muscle cells.

When high concentrations of adenosine are used, the greater depression of contractions to electrical stimulation as compared to norepinephrine is still evident. However, the inhibitory effect of the nucleoside on the sympathetic neurotransmission can no longer be demonstrated by measuring the outflow of labeled norepinephrine. In unstimulated strips, the same concentration of adenosine causes an increased efflux of ³H-norepinephrine and of deaminated metabolites; this suggests that more norepinephrine is leaking out of the storage vesicles into the neuronal cytoplasm, where it is deaminated by monoamine oxidase. This interpretation is supported by the experiments with pargyline, which further augments the decrease in ³H-norepinephrine caused by adenosine but reduces drastically the deaminated metabolites. In strips stimulated electrically, high concentrations of adenosine also cause an increased efflux of deaminated compounds, suggesting a similar increased leakage of norepinephrine from the vesicles. No such effect is observed with high concentrations of ATP; this may be related to the inability of nucleotides to cross the neuronal membrane. In contrast, high concentrations of ATP decrease the metabolites resulting from O-methylation, perhaps due to an inhibiting effect either on catechol-o-methyltransferase or on the extraneuronal binding or uptake of catecholamine.

Adenosine inhibits the release of ³H-norepinephrine evoked by nerve depolarization both with electrical impulses and potassium but not the displacement caused by tyramine. The liberation of norepinephrine by nerve depolarization is Ca²⁺-dependent, whereas that caused by tyramine is not. However, whether adenosine acts by hyperpolarizing the neuronal membrane, by directly inhibiting calcium influx, or by interfering in a further step in the excitation-secretion coupling cannot be answered by the present experiments. We can only speculate whether the antagonism by theophylline of the prejunctional effect of adenosine is an indication of the involvement of the cyclic nucleotide system in the inhibition of the sympathetic neurotransmission. The answer to this problem can only be provided by the development of methods to measure cyclic nucleotide levels in the sympathetic nerve endings in blood vessels.

The ability of adenosine and related nucleotides to inhibit release of norepinephrine in vascular strips implies that at least part of the vasodilator properties of these substances are due to such an effect on the sympathetic nerves. The experiments performed in intact dogs in which electrical stimulation was applied directly to the lumbar sympathetic chain yield results similar to those obtained in isolated blood vessels. These observations strengthen the conclusion that adenosine can interfere with adrenergic neurotransmission and indicate that this phenomenon can occur in vivo.

Evidence has accumulated in recent years that inhibition of adrenergic neurotransmission may represent a way of vasodilation shared by several naturally occurring vasoactive substances. Acetylcholine, α, ²¹ histamine, ²² and also moderate increases in potassium concentrations ²³ have been demonstrated to inhibit release of norepinephrine in canine blood vessels during nerve stimulation. The present studies extend this list of prejunctional vasodilators to adenosine and the adenine nucleotides. These substances are believed to play a role in the hyperemia of muscular exercise, ²⁰ a condition associated with decreased responsiveness to sympathetic stimulation. Activation of purinergic nerves has recently been considered to cause dilation of several adrenergically innervated blood vessels. ²² In these two situations the inhibitory effect of...
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purine compounds on adrenergic neurotransmission, as demonstrated in the present study, could play a role.

Acknowledgments

It is a pleasure to acknowledge the assistance provided in various portions of this study by R.R. Lorenz, M.D. Seppala, and J.Y. Troxell.

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Inhibition of sympathetic neurotransmission in canine blood vessels by adenosine and adenine nucleotides.
R H Verhaeghe, P M Vanhoutte and J T Shepherd

doi: 10.1161/01.RES.40.2.208

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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