Sympathetic Nervous System–Dependent Vasoconstriction in Humans

Evidence for Mechanistic Role of Endogenous Purine Compounds

Stefano Taddei, MD, Roberto Pedrinelli, MD, and Antonio Salvetti, MD

Purine compounds modulate sympathetic neurotransmission; this modulation decreases nervous discharge by stimulating presynaptic inhibitory adenosine receptors, an effect antagonized by theophylline, and causes vasoconstriction through the stimulation of postsynaptic ATP receptors. In humans we evaluated the effect of local theophylline, which was infused into the brachial artery at the rate of 100 \( \mu \text{g/100 cc/min} \), on the arteriolar sympathetic vasoconstriction induced by applying a lower-body negative pressure. Forearm blood flow changes were measured by strain-gauge venous plethysmography. Theophylline, which at this dosage blunted the vasodilator effect of adenosine (the physiological agonist for the P1 purinoceptor), significantly increased lower-body negative pressure–mediated vasoconstriction. To evaluate whether neurotransmitters different from norepinephrine participate in the vasoconstrictor effect of theophylline, we repeated the previous experiment in the presence of phenoxybenzamine, which was infused at a dose (60 \( \mu \text{g/100 cc/min} \)) that abolished the vasoconstrictor effect of norepinephrine. Also, after \( \alpha \)-adrenoceptor blockade, theophylline continued to increase sympathetic vasoconstriction. Our data confirm that purinergic receptors and neurotransmitters also participate in endogenous sympathetic vasoconstriction in humans. (Circulation 1990;82:2061–2067)

The classical concept that vasoconstriction induced by the stimulation of the sympathetic nervous system is mediated only by the activation of \( \alpha \)-adrenoceptors caused by the release of norepinephrine (NE) has been recently modified, since experimental data have shown that the vasoconstriction induced by sympathetic activation cannot be abolished by \( \alpha \)-adrenoceptor blockade.1

Recently, we have shown that forearm sympathetic vasoconstriction in humans cannot be abolished by complete \( \alpha \)-blockade, an effect that was accomplished by bretylium pretreatment.2 This finding is in agreement with evidence for the role of several different neurotransmitters and receptors in neural-dependent vasoconstriction found in experimental models.3–5

There is strong in vitro evidence that purine compounds modulate sympathetic vasoconstriction through the activation of specific receptors. As Burnstock6 proposed, purinoceptors can be separated into two different subclasses, \( P_1 \) and \( P_2 \). At the level of sympathetic neurovascular junctions, \( P_1 \) purinoceptors are located presynaptically; they are most sensitive to adenosine and AMP, and generally acting by means of an adenylylate cyclase system, they inhibit neurotransmitter release. On the contrary, \( P_2 \) purinoceptors are located postsynaptically; they are most sensitive to ATP and ADP and cause vasoconstriction not by means of adenylylate cyclase. Moreover, adenosine receptors have also been classified as \( A_1 \) and \( A_2 \),7 according to whether they inhibit or stimulate adenylylate cyclase, respectively. To avoid confusion due to the presence of these two classifications, in the present work purinoceptors will be named as adenosine (for \( P_1 \)) and ATP (for \( P_2 \)) receptors.

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During sympathetic stimulation, ATP is cosecreted with NE from adrenergic nerve endings and participates in vascular contraction.8–12 The release of both neurotransmitters is presynaptically inhibited by adenosine originating from rapid breakdown of ATP, and this effect is reversed by methylxanthines, such as theophylline, a selective antagonist for adenosine receptors.8,13–16

Therefore, we decided to evaluate whether adenosine receptor blockade also increases sympathetic va-
TABLE 1. Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>41.4±8.9</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>24/20</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>114.7±11.8</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>71.8±8.4</td>
</tr>
<tr>
<td>MIBP (mm Hg)</td>
<td>110.4±7.3</td>
</tr>
<tr>
<td>FBF (ml/min · dl)</td>
<td>3.6±0.9</td>
</tr>
<tr>
<td>FVR (ml · mm Hg/min · cc)</td>
<td>30.7±4.6</td>
</tr>
<tr>
<td>Forearm volume (ml)</td>
<td>913.4±101.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

MBP, mean blood pressure; HR, heart rate; MIBP, mean intra-arterial blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance.

soconstriction in humans and, if so, whether this effect was also partially dependent on the release of neurotransmitters other than NE. To test these possibilities, we studied the effect of local theophylline on forearm arteriolar vasoconstriction induced by endogenous sympathetic stimulation both in the absence and in the presence of complete α-adrenoceptor blockade.

Methods

Subjects

Forty-four patients (24 men and 20 women) aged 41.4±8.9 (mean±SD) years with mild to moderate, uncomplicated essential hypertension (see Table 1 for their characteristics) were recruited for the studies. According to institutional guidelines, all patients were aware of the investigational nature of the study and consented to it. All drugs were withdrawn for at least 1 week before the study, and patients abstained from dietary methylxanthines and alcohol for 48 hours before the study.

To avoid systemic hemodynamic modification or sympathetic nervous system reflexogenic activation, all drugs were infused into the brachial artery at systemically ineffective rates.

Endogenous sympathetic activation was obtained by a -10 mm Hg lower-body negative pressure (LBNP) for 5 minutes, which reduces venous return, deactivates cardiopulmonary inhibitory pathways, and causes sympathetic discharge and peripheral vasoconstriction without changes in systemic blood pressure and heart rate.

Experimental Procedure

All studies were performed in a quiet climatized room (22–24°C). A polyethylene cannula (21-gauge, Abbot, Sligo, Ireland) was inserted into the left brachial artery under light local anesthesia (2% lidocaine) and connected through stopcocks to a pressure transducer (model MS20, Electromedics, Englewood, Colo.) for systemic mean blood pressure (1/3 pulse pressure + diastolic pressure) and heart rate monitoring (model VSM1, Physiocontrol, Redmond, Wash.) and for intra-arterial infusions. The electronic beat-to-beat signal was digitized on line through an AT-IBM compatible personal computer, by using a customized software program (Sanware SNC, Casalecchio sul Reno, Bologna, Italy). A strain-gauge plethysmograph (LOOSCO, GL LOOS, Amsterdam, Holland) was used to measure forearm blood flow (FBF) at both limbs. Details concerning the sensitivity and the reproducibility of the method as performed in our laboratory have already been published.

Determination of forearm volume was performed according to the water displacement method. Drug infusion rates were normalized for 1 dl tissue by adjusting the speed of infusion to the desired infusion rates. Drugs were infused through separate ports with three-way stopcocks, as needed.

For the LBNP experiments, the inferior limbs were placed up to the waist into an airtight, Plexiglas container that was sealed by rubber flaps around the waist. Negative pressures, quantified by a U-shaped mercury manometer connected to the inside of the apparatus, were generated by means of a vacuum cleaner.

![Graph showing time course of forearm blood flow (FBF) during local infusion of cumulatively increasing doses of theophylline (100 µg/100 ml forearm tissue/min). Data are presented individually; the five symbols indicate five patients.](http://circ.ahajournals.org/doi/abs/10.1161/01.CIR.82.6.2062?journalCode=cir)
Experimental Design

In each study, effective local propranolol pre-treatment (10 μg/100 ml forearm tissue/min×15 min) was provided to avoid confounding interferences from β-adrenoceptor stimulation during sympathetic activation.

Effect of Local Theophylline on Adenosine-Mediated Vasodilation

To confirm in humans the antagonist properties of theophylline for adenosine purinoceptors, we evaluated its effect on forearm vasodilation induced by adenosine in a group of five patients. Adenosine, at three approximate log unit increments (0.5, 1.5, and 5.0 μg/100 ml forearm tissue/min×3 min each), was infused in the presence of saline (0.21 ml/min) and then infused in the presence of theophylline administered at a rate of 100 μg/100 ml forearm tissue/min×20 min. Since one of the mechanisms of action proposed for theophylline is phosphodiesterase inhibition, this dose was titrated in a preliminary experiment (n=5 subjects) as the highest one not affecting basal FBF, assuming vasodilation as the marker of phosphodiesterase activity inhibition (Figure 1).

Effect of Adenosine Receptor Blockade by Theophylline on Sympathetic Forearm Vasoconstriction

To test whether adenosine receptor blockade increases sympathetic activation, the forearm vasoconstriction induced by LBNP was evaluated in six patients in control conditions (saline, 0.2 ml/min) and after intra-arterial theophylline infusion at the rate of 100 μg/100 ml forearm tissue/min×20 min.

Effect of Theophylline on Sympathetic Forearm Vasoconstriction in Presence of a-Adrenoceptor Blockade

This series was based on the assumption that, if theophylline increases forearm vasoconstriction during sympathetic activation through NE overflow, a-blockade should abolish it. a-Adrenoceptor blockade was obtained through the irreversible antagonist phenoxybenzamine, which was infused into the brachial artery at 60 μg/100 ml forearm tissue/min for 60 minutes, a period of time sufficient for the full development of its effects. The adequacy of a-blockade was supported by our previous study and confirmed by a preliminary experiment (n=5 patients) in which phenoxybenzamine abolished the vasoconstrictor effect of a discrete a-adrenoceptor stimulation by intra-arterial NE infused at four cumulative rates (0.005, 0.015, 0.05, and 0.15 μg/100 ml forearm tissue/min×5 min each, in the presence of propranolol) (Figure 2).

The formal series was performed in a group of seven patients in whom LBNP was applied in the presence of saline and reapplied after the administration of phenoxybenzamine. Then LBNP was applied again during the infusion of theophylline, as well as phenoxybenzamine, at the previous rate.

Effect of Papaverine on Sympathetic Forearm Vasoconstriction in Presence of a-Adrenoceptor Blockade

To exclude the possibility that phosphodiesterase inhibition could also partially contribute to the effect of theophylline on the sympathetic stimulation, the previous protocol was repeated in another group of six patients by concurrently infusing, in the presence of phenoxybenzamine, papaverine, a selective phosphodiesterase activity inhibitor, at a rate of 25 μg/100 ml forearm tissue/min.

Analysis of Results

Since no relevant changes in mean arterial pressure took place during the study, all data were analyzed in terms of FBF. Wilcoxon’s test was used to check the statistical significance of the difference between mean values. Results were expressed as mean±SEM.

Drugs

Theophylline ethylendiamine (Tefamin, Recordati, Milan, Italy), adenosine (Sigma Chemical Co., Milan, Italy), papaverine (L.I.R.C.A. Synthelabo S.p.A., Milan, Italy), phenoxybenzamine HCl (Dibenzyline, Smith Kline and French, Welwyn Garden City, U.K.), propranolol HCl (Inderal, ICI, Milan, Italy), and (-)-norepinephrine bitartrate (Levophed, Breon, New York,) were obtained from commercially available

FIGURE 2. Graph showing abolition of phenoxybenzamine (60 μg/100 ml forearm tissue/min×60 min) of dose-dependent forearm vasoconstrictor effect of norepinephrine (N.E) in presence of propranolol (10 μg/100 ml forearm tissue/min×15 min). The effect of norepinephrine is calculated as percent forearm blood flow (FBF) decrements from basal. Data are mean±27 (n=5).
sources and diluted in fresh solutions to the desired concentrations by adding normal saline.

Results
Effect of Local Theophylline on Adenosine-Mediated Vasodilation

In control conditions (saline), adenosine infusion caused a dose-dependent vasodilation. When repeated in the presence of theophylline, its effect was abolished at the first two infusion rates and strongly reduced at the higher one (Figure 3). When the data are expressed as percent changes from basal FBF, it appears that theophylline shifts the dose-response curve to adenosine significantly to the right (saline: +28.8±11.3%, +103.9±26.7%, and +217.4±29.7%; theophylline: +1.0±2.1%, +13.3±7.6%, and +65.4±38.5%; p<0.001).

Effect of Adenosine Receptor Blockade by Theophylline on Sympathetic Forearm Vasoconstriction

In control conditions, LBNP caused a significant (p<0.001) vasoconstriction in both forearms that was significantly greater in the experimental forearm (FBF from 3.6±0.7 to 2.2±0.6 ml/100 cc tissue/min [experimental] compared with FBF from 3.6±0.8 to 2.6±0.5 ml/100 cc tissue/min [control]), owing to the presence of β-blockade induced by propranolol. When the stimulus was repeated in the presence of theophylline, which, per se, did not affect basal FBF, LBNP-mediated vasoconstriction remained unchanged in the control forearm but was significantly greater (p<0.001) than before theophylline infusion in the experimental forearm (FBF from 3.7±0.7 to 1.7±0.4 ml/100 cc tissue/min) (Figure 4). When data are reported as percent FBF decrements with respect to basal values, theophylline significantly increases the vasoconstrictor effect of LBNP (saline: −37.6±4.8%; theophylline: −52.8±7.4%; p<0.01).

Effect of Theophylline on Sympathetic Forearm Vasoconstriction in the Presence of α-Adrenergic Blockade

Confirming our previous data,2 phenoxybenzamine did not abolish LBNP-mediated vasoconstriction
(FBF from 9.6±1.4 to 8.5±1.4 ml/100 cc/min; −10.9±4.4%) but only reduced it compared with basal (FBF from 3.9±0.7 to 2.7±0.4 ml/100 cc/min; −34.7±5.3%) (Figure 5). When theophylline was overinfused and LBNP was applied again, the vasoconstrictor effect of the endogenous stimulus significantly (p<0.01) increased (FBF from 9.6±1.3 to 7.7±1.1 ml/100 cc/min; −20.2±4.9%) (Figure 5). In the control forearm, LBNP-induced vasoconstriction did not change, excluding nonspecific time-dependent modifications.

**Effect of Papaverine on Sympathetic Forearm Vasoconstriction in the Presence of α-Adrenoceptor Blockade**

As in the previous study, phenoxybenzamine reduced but did not abolish the LBNP-mediated vasoconstrictor effect (FBF from 3.4±0.8 to 2.1±0.6 ml/100 cc/min [basal] versus FBF from 8.8±1.7 to 7.1±1.3 ml/100 cc/min [phenoxybenzamine]; −37.4±11.9% versus 17.2±4.1%, respectively). When papaverine was overinfused to phenoxybenzamine, it caused a further increment of FBF (from 8.6±1.3 to 10.1±2.3 ml/100 cc/min). When the LBNP application was repeated, the amount of sympathetic vasoconstriction residual to α-blockade was reduced (FBF from 10.1±2.3 to 8.6±1.6 ml/100 cc/min; −14.8±4.4%; p<0.001).

**Discussion**

The present study shows that in humans theophylline increases endogenous sympathetic vasoconstriction obtained by applying LBNP and that this effect is also persistent in the presence of complete α-adrenoceptor blockade.

These data are in agreement with in vitro studies suggesting that theophylline modulates sympathetic activation through an increased neurosecretion of NE and of other purine compounds, possibly ATP.

With regard to the mechanism of this effect, theophylline might act either as an antagonist of adenosine receptors or as an inhibitor of phosphodiesterase activity, with the consequent accumulation of cyclic AMP. However, it is possible to dissect these two effects, since the antagonism for adenosine appears at a plasma concentration lower than that needed for phosphodiesterase inhibition. Therefore, we infused a dose of theophylline that did not increase basal FBF, suggesting a lack of inhibition of phosphodiesterase activity. Otherwise, at the same rate, the drug blunted adenosine-induced vasodilation, an effect mediated by adenosine receptor antagonism. Moreover, although the increment of cyclic AMP has been reported to increase NE release from the vas deferens and the spleen of rats, this effect seems to be very small. As a consequence, it is possible to affirm that, in our experimental conditions, the increment of the vasoconstrictor effect of an endogenous sympathetic stimulus induced by theophylline was determined by the antagonism for adenosine receptors. Therefore, these data are consistent with the view that endogenous adenosine also plays a modulating role in sympathetic neurotransmission in humans.

According to the experimental data, the potentiating effect of theophylline is attributed to neurotransmitter overflow. To investigate whether neurotransmitters other than NE might also contribute to purinergic modulation in humans, we performed the experiments with phenoxybenzamine. Our working hypothesis was based on the assumption that, if NE were the only neuromediator involved in the theophylline-mediated potentiating effect on sympathetic vasoconstriction, α-blockade should abolish it. The effectiveness of local phenoxybenzamine at high concentrations in determining a complete α-adrenoceptor blockade in our experimental conditions was already discussed and also confirmed further by the abolition of the forearm vasoconstriction induced by
a discrete \(\alpha\)-adrenoceptor stimulation through NE at increasing doses over a 30-fold range (Figure 2). At variance with NE, the vasoconstrictor effect of LBNP is reduced but not abolished by phenoxybenzamine, further confirming the presence of a certain amount of sympathetic vasoconstriction resistant to \(\alpha\)-adrenoceptor blockade.\(^2\) When theophylline was superimposed on phenoxybenzamine, the residual vasoconstriction to LBNP application was increased, suggesting the involvement of neurotransmitters operating through the stimulation of receptors different from \(\alpha\)-adrenoceptors. On the contrary, in the same experimental conditions, papaverine reduced the amount of sympathetic vasoconstriction resistant to phenoxybenzamine; this fact confirms that this effect of theophylline is also independent of any eventual inhibitory action of phosphodiesterase and points to an antagonistic action on adenosine receptors.\(^{26–28}\) Taken together, these data show that purinergic modulation also operates through mechanisms different from NE release-mediated \(\alpha\)-adrenoceptor stimulation, suggesting the involvement of different neurotransmitters, such as ATP or others.

Another possible interpretation of these data might be that theophylline could antagonize the vasodilating effect of local adenosine present in blood or released by endothelial cells.\(^32\) Though we cannot directly exclude this hypothesis, evidence exists that in the basal condition the level of purines in the blood is low,\(^33\) as confirmed by the lack of effect of theophylline on basal FBF. On the contrary, the local release of purine compounds becomes important in particular conditions such as hypoxia or exercise.\(^33\) The sympathetic stimulus that we used is quite selective\(^17\) and devoid of systemic hemodynamic modifications.\(^18\) Thus, it is highly unlikely that extraneural sources of purines play an important role in our experimental conditions.

In conclusion, our data seem also to confirm a physiological role in humans for the purinergic system in the regulation of vascular tone in forearm arterioles during sympathetic nervous system activation. The results are also consistent with, and add further weight to, the opinion that sympathetic vasoconstrictor mechanisms that are not dependent on \(\alpha\)-adrenoceptor stimulation play a role in neurogenic vasoconstriction.

Acknowledgments

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References


KEY WORDS • norepinephrine • vasoconstriction • theophylline • phenoxybenzamine • α-blockade • forearm blood flow
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S Taddei, R Pedrinelli and A Salvetti

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