Antagonistic Effects of Theophylline and Adenosine on Adrenergic Neuroeffector Transmission in the Rabbit Kidney

PER HEDQVIST, BERTIL B. FREDHOLM, AND SIW ÖLUNDH

SUMMARY The actions of adenosine and theophylline on adrenergic neuroeffector transmission were studied in the rabbit kidney perfused with Tyrode's solution in which the norepinephrine stores had been labeled with (-)-norepinephrine[3H] ((−)-NE[3H]). We found that adenosine inhibited (-)-NE[3H] release induced by nerve stimulation, increased basal perfusion pressure, and enhanced the vasoconstrictor response to nerve stimulation and norepinephrine in a dose-dependent manner. Theophylline per se had effects on neuroeffector transmission opposite to those of adenosine. All effects of adenosine were antagonized effectively or annulled by theophylline in concentrations having little or no effect on rabbit kidney phosphodiesterase activities. Two other compounds, Ro 20-1724 and ZK 62.711, being equally potent or more potent than theophylline as phosphodiesterase inhibitors, failed to antagonize adenosine-mediated inhibition of (-)-NE[3H] release by nerve stimulation. Ro 20-1724 in high concentration (10⁻⁴ m) inhibited the vasoconstrictor response to nerve stimulation, but it had little additional effect on the enhancement by adenosine. These findings suggest that theophylline specifically antagonizes the effects of adenosine on pre- and postjunctional transmission in the kidney. The results also are consistent with the view that endogenous adenosine may play a role as modulator of adrenergic neuroeffector transmission.

RECENTLY it was shown that adenosine causes a dose-dependent and reversible inhibition of norepinephrine (NE) release evoked by nerve impulses in the rabbit kidney, canine subcutaneous adipose tissue, and guinea pig vas deferens. Although adenosine depressed transmitter release, it caused enhancement of vasoconstrictor responses to nerve stimulation in the kidney. This was shown to be due to a postjunctional potentiation of the effects of administered NE, in agreement with earlier findings in the dog. Adenosine is a potent vasodilator in most vascular beds, but it causes vasoconstriction in the kidney. Independently of whether adenosine is vasoconstrictor or vasodilator, its vascular effects seem to be competitively antagonized by theophylline. Several other effects of adenosine, such as enhanced cyclic AMP accumulation in brain slices, inhibition of cerebral cortical neurons, inhibition of cyclic AMP accumulation in fat cells and enhancement of contractility in vas deferens, also are inhibited by theophylline.

The present investigation was undertaken to assess the capacity of theophylline to antagonize the modulating actions of adenosine on adrenergic neuroeffector transmission in the rabbit kidney. Data are presented indicating that theophylline, in concentrations having little or no effect on kidney phosphodiesterase activities, antagonizes both the inhibitory and stimulant effects of adenosine. A preliminary account of some of the results has been presented elsewhere.

Methods

Rabbits of either sex, weighing 2–3 kg, were anesthetized with sodium pentobarbital, 40–50 mg/kg, iv. The left kidney, with its nervous and vascular supply, was dissected free from surrounding tissue. After administration of heparin (1000 IU/kg body weight), the vessels and the ureter were cannulated and the kidney was flushed with ice-cold, O₂-gassed 0.9% NaCl, containing 50 IU of heparin per ml. The preparation was then transferred to a perfusion chamber, and perfused with Tyrode's solution (concentration in mM: NaCl, 136.7; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 1.0; NaHCO₃, 11.0; NaH₂PO₄, 0.4; glucose, 5.5; ascorbic acid, 0.1), containing 2% dextran and gassed with 5% CO₂ and O₂, at 37°C. Perfusion flow was kept constant at a rate of 10 ml/min and pressure was recorded arterially, close to the kidney, with a Statham (P23 AC) pressure transducer and a Grass polygraph.

The nerve was placed on platinum wire electrodes and stimulated with a Grass S4 stimulator, delivering 15-sec trains of pulses (5–6 Hz, 1 msec, 10 V) at intervals usually of 10 minutes. The NE stores were labeled by a 40-minute infusion of 50 μCi of (−)-NE[3H] (specific activity, 5.4 Ci/mmol; New England Nuclear) in 0.9% NaCl. Before the experiment was started, the kidney was perfused with isotope-free Tyrode’s for 30–45 min-
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FIGURE 1 Effects of theophylline and adenosine on (-)-NA$[^3]$H release and effector responses to sympathetic nerve stimulation (6 Hz, 90 pulses at arrows) in Tyrode-perfused (10 ml/min) rabbit kidney.

The following drugs were used: adenosine and norepinephrine bitartrate (Sigma), 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) (Hoffman-La-Roche AG), theophylline ethylenediamine (ACO, Stockholm), and 4-(3-cyclopentyl-oxy-4-methoxyphenyl)-2-pyrrolidone (ZK 62.711) (Schering AG). All drugs were diluted in 0.9% NaCl and administered by close arterial injection. Concentrations of administered drugs refer to the base.

The perfusate was collected in 1-minute fractions (10 ml) and the radioactivity was determined by counting 1.0-ml samples in 10 ml of Instagel (Packard Instruments) in a Packard liquid scintillation spectrometer. The values were expressed as counts/min. Quenching was monitored by external standardization.

Cyclic nucleotide phosphodiesterase (EC 3.1.4.17c) was determined, essentially, according to the method of Selstam and Rosberg, as described elsewhere, using $^{32}$P-labeled adenosine 3',5'-cyclic monophosphate triethylammonium salt (specific activity, 20-33 Ci/mmol; New England Nuclear) as substrate, and separation of products and substrate by inorganic salt precipitation with ZnSO$_4$-Ba(OH)$_2$. Kidney cortex was homogenized in 80 mM Tris-Cl (pH 7.8) containing 7 mM 2-mercaptoethanol. Final cyclic AMP concentration was 0.2 $\mu$mol/l. The assay was performed at 30°C for 10 minutes, using enzyme diluted so that less than 20% of the substrate was converted. The assay was linear with time up to 40 minutes and with the amount of enzyme protein. Blank values corresponded to 8% conversion of cyclic AMP with a coefficient of variation less than 0.4% of that value. Radioactivity was determined both in the supernatant extract and pellet using Cerenkovradiation. Results are given as IC$_{25}$ and IC$_{50}$ based on triplicate determinations at five or more different concentrations of inhibitor.

Statistics

Experimental data were expressed as means ± SE. Statistical hypotheses were tested by Student’s $t$-test for paired and unpaired variates, and by linear regression analysis.

Results

In confirmation of our previous report, it was noted that adenosine (10$^{-6}$ M) consistently and reversibly depressed the release of (-)-NE$[^3]$H in response to nerve stimulation (15-sec trains of pulses at 5 Hz) in the Tyrode-perfused rabbit kidney. In this concentration, adenosine also increased basal perfusion pressure and enhanced the vasoconstrictor responses. The potentiation of vasoconstrictor responses showed marked variability, ranging from a few to more than 100 mm Hg. There was no statistically significant correlation between enhancement of the vasoconstrictor response and inhibition of (-)-NE$[^3]$H release ($r = -0.29, \text{n} = 21, P > 0.1$).

Intravenous infusion of theophylline (10$^{-6}$-10$^{-4}$ M) had no discernible effect on spontaneous release of tracer or basal perfusion pressure. On the other hand, the compound was found to increase the release of (-)-NE$[^3]$H and to depress vasoconstriction induced by nerve stimulation (Fig. 1). Both

Note that the abbreviation "NA" in all figures and figure legends refers to "noradrenaline," which where feasible has been changed, in accord with U.S. usage, to read "NE" or "norepinephrine."

Drug concentration causing 25% and 50% inhibition, respectively.
effects were rapid in onset and disappeared quickly after the infusion of theophylline. Enhancement of \((-\text{-NE}[^{3}H])\) release was not a very prominent phenomenon but reached the level of statistical significance with $10^{-2}$-10^{-4}\text{ M}$ theophylline (Fig. 2). In these concentrations, theophylline inhibited markedly and dose dependently the vasoconstrictor response to nerve stimulation, as well as that to injection of NE (0.4\text{ \textmu g}) (Figs. 2, 3).

Theophylline (10^{-5}-10^{-4}\text{ M}) markedly and dose dependently depressed adenosine (10^{-6}\text{ M}) enhanced vasoconstrictor responses to both nerve stimulation and injected NE (Fig. 4). At 10^{-5}\text{ M} theophylline, the antagonism resulted in vasoconstrictor responses close to the control level, i.e., responses obtained in the absence of adenosine and theophylline; 10^{-4}\text{ M} theophylline abolished the enhancing effect of adenosine (10^{-6}\text{ M}), as witnessed by the finding that the vasoconstrictor responses were as small as those obtained in the presence of theophylline alone. The direct pressor effect of adenosine (10^{-6}\text{ M}) was markedly reduced already by 10^{-6}\text{ M} theophylline (Fig. 1).

Theophylline also counteracted effectively the inhibitory effect of adenosine (10^{-6}\text{ M}) on \((-\text{-NE}[^{3}H])\) release induced by nerve stimulation. This is perhaps surprising in view of the relatively small effects of theophylline per se on this parameter. Nevertheless, theophylline (10^{-5}\text{ M}) partially removed the inhibition by adenosine (10^{-6}\text{ M}) on \((-\text{-NE}[^{3}H])\) release. At 10^{-4}\text{ M} theophylline nullified the inhibitory effect of adenosine (10^{-6}\text{ M}), leading to \((-\text{-NE}[^{3}H])\) output figures statistically indistinguishable from those obtained with nerve stimulation in the presence of theophylline alone (Fig. 5). Raising the adenosine concentration to

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure2}
\caption{Effects of theophylline on \((-\text{-NA}[^{3}H])\) release and vasoconstriction induced by nerve stimulation (6 Hz, 90 pulses). Data presented in % of a preceding control stimulation and given as means ± se. Numbers in parentheses = number of experiments. \*\*\*P < 0.01.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure3}
\caption{Effects of theophylline on adenosine-enhanced vasoconstriction in response to nerve stimulation (6 Hz, 90 pulses) and NA (0.4 \textmu g). Data presented in % of a control stimulation in the absence of adenosine and theophylline. Symbols to the left denote enhancing effect of adenosine. Mean values ± se. Numbers in parentheses = number of experiments. \*\*\*\*P < 0.001.}
\end{figure}

10^{-5}\text{ M} surmounted the antagonistic effect of theophylline. In these cases the inhibition of \((-\text{-NE}[^{3}H])\) release was unaffected by 10^{-5}\text{ M} theophylline and only partially antagonized by the 10 times higher concentration.

Since theophylline is known as a phosphodiesterase inhibitor, two other phosphodiesterase inhibitors, Ro 20-1724 and ZK 62.711, were tested for effects on adenosine-mediated modulation of adrenergic neurotransmission in the kidney. Neither Ro 20-1724 (10^{-6}-10^{-4}\text{ M}) nor ZK 62.711 (10^{-6}-10^{-5}\text{ M}) affected the inhibitory effect of adenosine (10^{-6}\text{ M}) on release of \((-\text{-NE}[^{3}H])\) induced by nerve stimu-
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Vasoconstriction

**NA** Release

**THEOPHYLLINE** (molar conc.)

**FIGURE 5** Effects of Theophylline-adenosine interaction on (-)-NA[^3H] release and vasoconstrictor responses to nerve stimulation (6 Hz, 90 pulses). Data presented in % of a preceding control stimulation in the absence of adenosine and theophylline. Symbols to the left denote effects of adenosine alone on responses to nerve stimulation. Mean values ± SE. Numbers in parentheses = number of experiments. **P < 0.01, **P < 0.001.

Adenosine Enhanced Vasoconstrictor Response (Figs. 6, 7). In these concentrations the two compounds per se were devoid of action on nerve-induced release of (-)-NE[^3H] (Figs. 6, 7). The adenosine-enhanced vasoconstrictor response to nerve stimulation was reduced only by 10^-4 M Ro 20-1724. However, at this concentration, Ro 20-1724 per se inhibited the vasoconstrictor response, and the response was significantly higher in the presence of both adenosine and Ro 20-1724 than with Ro 20-1724 alone (Table 1). To establish whether Ro 20-1724 actually antagonized the effect of adenosine, the net effect of adenosine (10^-6 M) on vasoconstriction and (-)-NE[^3H] release in the presence of Ro 20-1724 was calculated (Fig. 8). It can be seen that the effect of adenosine on (-)-NE[^3H] release was unaffected, and that on the vasoconstrictor response diminished less than 10% by Ro 20-1724 (10^-4 M). On the other hand, the effects of adenosine on both (-)-NE[^3H] release and vasoconstrictor response were effectively and similarly antagonized by theophylline, the calculated IC50 being 25 and 14 μM, respectively (Fig. 8).

The phosphodiesterase-inhibiting effect of theophylline, Ro 20-1724, and ZK 62.711 was studied in homogenates of rabbit kidney cortex. At 10^-5 M the compounds gave little or no inhibition of cyclic AMP phosphodiesterase. Drug concentrations ranging from 0.49 to 0.60 mM were required to produce a 50% inhibition of 5'-AMP[^32P] formation from cyclic AMP[^32P] (Table 2). The three compounds are equally or less active on cyclic GMP phosphodiesterase in the rabbit kidney.

**TABLE 1** Effects of Adenosine (10^-6 M) and Ro 20-1724 (10^-4 M), Alone and in Combination, on Vasoconstrictor Responses to Renal Nerve Stimulation (6 Hz, 90 Pulses).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Adenosine</th>
<th>Ro 20-1724</th>
<th>Ro 20-1724</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(10^-6 M)</td>
<td>(10^-4 M)</td>
<td>(10^-4 M)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>106.6 ± 9.6</td>
<td>67.0 ± 4.2</td>
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<td>(P &lt; 0.01)</td>
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<td>100</td>
<td>166.8 ± 14.5</td>
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<td></td>
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<td>(P &lt; 0.01)</td>
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</table>

Data presented in % of a preceding control stimulation. Mean values ± se; n = 6. P values within parentheses denote difference from control. NS = not significant.
Discussion

In the present study the NE stores in the rabbit kidney were labeled with (−)-NE[^3H] and outflow of tracer was used as an index of transmitter release. Previously we have shown that 81% of the radioactivity overflowing from the kidney during nerve stimulation is accounted for by intact (−)-NE[^3H]. Adenosine decreased and theophylline increased total overflow of radioactivity during nerve stimulation, without affecting basal outflow of radioactivity. This indicates that adenosine decreased and theophylline increased the release of transmitter in response to nerve stimulation. The possibility that the two compounds might in addition have altered the metabolic degradation of released (−)-NE[^3H] was not specifically looked for. It has been shown, however, that adenosine, within the concentration range used in the present study, does not affect the enzymatic degradation of (−)-NE[^3H] in the perfused canine saphenous vein.

The major finding of the present study is that theophylline antagonized the inhibitory effect of adenosine on NE release as well as its enhancing effect on vasoconstriction induced by nerve stimulation and administered NE. Since these effects of theophylline were dose dependent, reversible, and surmounted by raising the adenosine concentration, a competitive antagonism is indicated. In addition, theophylline antagonized the direct pressor effect of adenosine, which is in keeping with observations in dog and rat kidney.

Previously we have found that the prejunctional effect of adenosine in the rabbit kidney is unaffected by drugs that block prostaglandin synthetase or the α-adrenoceptors. Since α-adrenoceptor blockade completely inhibits vasoconstrictor responses to nerve stimulation and NE, it is unlikely that inhibition of NE release by adenosine is secondary to its enhancing effect on the vasoconstrictor responses. The present observation that there was no significant correlation between adenosine-mediated enhancement of the vasoconstrictor response and inhibition of NE release further stresses the opinion that the two effects are independent.

Theophylline is a phosphodiesterase inhibitor and hence may cause increased levels of cyclic AMP. Both methylxanthines and dibutyryl cyclic AMP have been reported to increase catecholamine secretion from the adrenal medulla and NE release from vas deferens and spleen, although in the vas deferens the effect has been considered very small. In the present study, theophylline concentrations having little or no effect on kidney phosphodiesterase antagonized the effects of adenosine on perfusion pressure and adrenergic neuroeffector transmission, and abolition of the adenosine effects was obtained with theophylline in a concentration producing no more than 25% inhibition of phosphodiesterase. Moreover, Ro 20-1724 and ZK 62.711, equal or more potent phosphodiesterase inhibitors than theophylline in several tissues, including rabbit kidney, (present results), were found to be devoid of action on the adenosine-mediated inhibition of NE release. Notably, Ro 20-1724 (10⁻⁴ M) depressed the adenosine-enhanced vasoconstrictor response to nerve stimulation. However, Ro 20-1724 per se inhibited the vasoconstrictor response, and the net effect of adenosine was largely unaffected by Ro 20-1724 (cf. Fig. 8). It may be concluded, therefore, that the observed effects of theophylline cannot be explained in terms of phosphodiesterase inhibition. On the other hand, the results are compatible with the opinion that theophylline specifically interacts with effects of adenosine on neuroeffector transmission in the kidney. It has been proposed that theophylline is an antagonist acting on an adenosine receptor located on the cell surface. The present finding that theophylline antagonized the pre- and postjunctional effects of adenosine in an almost parallel manner (cf. Fig. 8) suggests the presence of such adenosine receptors of similar type and sensitivity on the membrane of both the nerve terminal and the effector cell.

Table 2. Molar Concentrations of Theophylline, Ro 20-1724, and ZK 62.711 Caused 25% and 50% Inhibition of Phosphodiesterase Activity in Rabbit Kidney Cortex Homogenates

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC₅₀</th>
<th>IC₉₀</th>
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<tbody>
<tr>
<td>Theophylline</td>
<td>1.2 × 10⁻⁴</td>
<td>6.0 × 10⁻⁴</td>
</tr>
<tr>
<td>Ro 20-1724</td>
<td>1.0 × 10⁻⁴</td>
<td>6.0 × 10⁻⁴</td>
</tr>
<tr>
<td>ZK 62.711</td>
<td>6.6 × 10⁻⁵</td>
<td>4.9 × 10⁻⁴</td>
</tr>
</tbody>
</table>
The observed interactions of theophylline and adenosine on adrenergic neuroeffector transmission raises the intriguing question of whether the effects of theophylline per se are due to an antagonism of endogenous adenosine. Elsewhere we have reported that nerve stimulation causes release of adenosine, mostly of postjunctional origin, in the rabbit kidney. 29-30 It is likely, however, that adenosine released from postjunctional cells also can reach the nerve terminals, possibly in concentrations sufficient to affect NE release. Furthermore, it is conceivable that purines such as adenosine may be released directly from nerves by nerve stimulation. 31 Theophylline and adenosine have opposite effects on NE release and vasconstrictor responses to nerve stimulation, and theophylline readily antagonizes both the pre- and postjunctional effects of adenosine. It is possible, therefore, that the effects of theophylline per se on NE release and vasconstric- tor responses to nerve stimulation reflect, at least in part, an antagonism of endogenous adenosine.

According to results of experiments on dogs and rats, adenosine administration is associated with increased vascular resistance in the kidney, a reduction in renal blood flow and glomerular filtration rate, and a decreased sodium excretion, all effects which are effectively blunted by theophylline. 6, 10, 21-23, 32-34 Also the tubulo-glomerular feedback control of filtration rate in rats is antagonized by methylxanthines, possibly as a result of inhibition of endogenous adenosine. It is possible, therefore, that the effects of theophylline on NE release and vasoconstrictor responses to nerve stimulation reflect, at least in part, an antagonism of endogenous adenosine.

The present results, showing that theophylline effectively, and presumably specifically, blunts both the pre- and postjunctional effects of adenosine on transmission, and that it produces effects opposite to those of adenosine, clearly add one more significant level of theophylline-adenosine interaction in the kidney. The results are also consistent with, and add further weight to, the opinion that adenosine may play a role as modulator of adrenergic neuroeffector transmission. 1

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Contribution of Systemic Venous Hypertension to the Development of Pulmonary Edema in Dogs

WARREN C. MILLER, WARREN W. SIMI, AND DAVID L. RICE

SUMMARY Systemic venous hypertension (SVH) is a frequent finding in pulmonary edema. To study the possible contributory or even causal role of SVH in pulmonary edema, a dog model was developed in which balloon catheters were placed in the left and right atria. Inflation of the left atrial balloon produced a tendency to pulmonary edema by causing pulmonary venous hypertension (PVH) (pulmonary artery wedge pressure of 20 mm Hg). Inflation of the right atrial balloon produced SVH (central venous pressure of 15 mm Hg). After 2 hours, dogs with SVH with or without PVH demonstrated a greater amount of lung fluid accumulation (P < 0.01) compared to controls or PVH alone. There was no significant difference in lung water in SVH dogs with or without PVH. Pulmonary blood flow was not significantly different between the experimental groups, each of which was less than control. Impairment of pulmonary lymphatic flow is one possible mechanism producing the worsening edema; however, bronchial venous hypertension or neurogenic reflexes cannot be excluded. We conclude that the contribution of systemic venous hypertension to the development of pulmonary edema may have therapeutic implications.

INCREASED extravascular lung water (pulmonary edema) has been demonstrated in humans with cor pulmonale, a condition characterized by systemic venous hypertension (SVH), rather than the pulmonary venous hypertension (PVH) seen in classic cardiogenic pulmonary edema due to left heart failure. The latter may additionally show systemic venous hypertension recognized by the clinician as distended neck veins, hepatic congestion and edema of the extremities. Various forms of noncardiogenic pulmonary edema also are accompanied by elevation of central venous pressure (CVP) which is independent of the level of pulmonary venous pressure. It has been postulated that the lung fluid accumulation seen in cor pulmonale is a consequence of SVH which produces back-pressure on bronchial veins that leads to pulmonary edema. Because of the frequent association of an elevated CVP and pulmonary edema and the suggestions that it may play a primary causal role, we have examined the contribution of systemic venous hypertension to the development of pulmonary edema in a canine model.

Methods

Healthy mongrel dogs weighing 22.5-29.3 kg were anesthetized with intravenous pentobarbital (30 mg/kg, iv), secured in a supine position, intubated and ventilated with a volume ventilator (Harvard Apparatus). Control of CVP in the upper body was obtained by variable inflation of a balloon catheter placed at the junction of the superior vena cava and right atrium via a jugular vein. Pulmonary capillary wedge pressure (PCWP), was elevated by a balloon catheter placed directly in the left atrium through a left thoracotomy; the incision was closed airtight
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