Pharmacological Evidence for A₁ and A₂ Adenosine Receptors in the Skin Microcirculation

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To characterize adenosine-mediated vascular responses, synthetic A₁ and A₂ receptor agonists (N-ethyl carboxamido adenosine [NECA], 2-chloro adenosine [2CA], or cyclohexyl adenosine [CHA]), the parent compound (adenosine [ADO]), an uptake inhibitor (dipyridamole [DIPYRID]) or a nonselective, competitive antagonist (8-phenyl theophylline [8pTHEO]) were topically applied to 20–60 μm arterioles in the subcutaneous microcirculation of the hamster. Blood flow was calculated from arteriolar diameter and red blood cell velocity using intravital microscopy. At >10⁻⁴ M, the potency order for vasodilation (maximum, 170–190% of control) was NECA > 2CA > ADO; these responses were attenuated by 10⁻³ M 8pTHEO. From 10⁻⁸ to 10⁻⁴ M, 2CA evoked vasodilation whereas ADO, which has an identical affinity at A₁ and A₂ receptors, evoked lesser responses. ADO-induced vasodilation was potentiated by 10⁻⁵ M DIPYRID; this response was similar to that evoked by 2CA alone or 2CA+DIPYRID. In contrast to ADO, 2CA is a poor substrate for cellular uptake, which suggests that uptake reduces the A₂ effect of exogenous ADO. From 10⁻¹⁰ to 10⁻⁸ M, CHA and ADO were equipotent for causing vasoconstriction (minimum, 80–90% of control); these responses were completely antagonized by 8pTHEO. Norepinephrine was a more potent vasoconstrictor and 8pTHEO did not alter these responses. Since ADO is a metabolic substrate and a nonselective receptor agonist, while CHA is A₁-selective and a poor substrate for cellular uptake, neither A₁ activation nor cellular uptake altered expression of the A₁ effect of exogenous ADO. Furthermore, DIPYRID had no effect on the A₁ response. Thus, depending on agonist concentration, stimulation of high affinity A₁ receptors or low affinity A₂ receptors can cause opposite responses in the skin microcirculation. The precise location or physiological role of these receptors is unknown. (Circulation Research 1989;65:176–184)

Adenosine has many actions that could influence skin blood flow, including neuromodulation, direct or indirect vascular smooth muscle effects, and alteration of leukocyte function. The physiological role of this nucleoside in the skin microcirculation probably depends on the amount and source of extracellular adenosine and on the type and distribution of purine receptors. However, no previous studies have identified adenosine receptors in the cutaneous microcirculation. In several tissues, two classes of extracellular adenosine receptors have been defined by the effects of various synthetic analogues on adenylate cyclase: at A₁ receptors, the potency for inhibiting adenylate cyclase is in the nanomolar range in various systems, and the potency order is cyclohexyl adenosine (CHA) > 2-chloro adenosine (2CA) > adenosine (ADO) > N-ethyl carboxamido adenosine (NECA); at A₂ receptors, the potency is in the micromolar range, the order is reversed, and adenylate cyclase is stimulated. Paradoxically, adenosine itself cannot be used to differentiate between receptor-mediated and non-receptor-mediated responses because it is a metabolic substrate as well as a nonselective receptor agonist. Furthermore, new evidence suggests that adenosine receptors may not be tightly linked to adenylate cyclase. The purpose of this study was to pharmacologically characterize adenosine-mediated responses in the skin microcirculation. It was assumed that adenosine receptor stimulation would elicit parallel changes in arteriolar diameter because adeno-
sine receptor stimulation elicits parallel changes in adenylylate cyclase and blood flow in the heart, kidney, and brain.3,4,13

Materials and Methods

General

Male Syrian golden hamsters (100–140 g) were premedicated with atropine (3 μg/100 g i.m.) and anesthetized with sodium pentobarbital (10–20 mg/100 g i.p.; Nembutal, Abbott Laboratories, Abbott Park, Illinois). The trachea was cannulated, and respiration was spontaneous on room air supplemented with O2. Rectal temperature was monitored continuously (model 72, YSI, Yellow Springs, Ohio) and maintained at 36–38°C by a heat lamp. Catheters were placed in a femoral vein and artery. A solution of pentobarbital in saline (10 mg/ml) was continuously administered through the vein (0.5 ml/hr) to maintain anesthesia and to supplement surgical and evaporative fluid losses. Systemic blood pressure was continuously measured through the arterial catheter and ranged between 80–120 mm Hg during the 3–6-hour duration of the typical experiment. An experiment was terminated if arterial blood pressure decreased below 70 mm Hg.

The skin microcirculation was prepared as previously described.14 Briefly, the animal was positioned laterally on a specially designed Plexiglas animal board. The dorsal skin along the midline was elevated from the back and secured to the board with silk sutures. A 1.5 cm diameter section of skin was dissected and removed from the subcutaneous tissue underlying the intact epidermis on the opposite side. All bleeding points were ligated. The surrounding tissue lying on the blood vessels was dissected and removed from the exposed area.

Throughout the surgical and experimental procedures, the tissue was suffused with a bicarbonate-buffered Ringer’s solution consisting of (mM) NaCl 131.9, KCl 4.7, CaCl2·2H2O 2.0, MgSO4·7H2O 1.2, and NaHCO3 20.0. Solution temperatures, gas tensions, and pH were maintained by vigorous bubbling with a gaseous mixture of 5% CO2-95% N2 in glass chambers encased in water jackets at 37°C. Tissue temperature was continuously monitored and maintained between 31–33°C by varying the flow rate (1–5 ml/min) of the heated suffusate solution.

The tissue was transilluminated with white light from a 50 W mercury lamp with a heat absorption filter in series with the light source. Observations were made with a microscope (Leitz Laborlux D, Rockleigh, New Jersey) equipped with a long working distance (4–6 mm) objective (Leitz L-20X, 0.32 n.a.) and a long working distance condensor (Leitz L-35, 0.30 n.a.). The preparation was visualized with a video camera (model 66, Dage-MTI, newvicon tube; Michigan City, Indiana) that transmitted a signal to a video analyzer (model 321, Colorado Video, Boulder, Colorado) and a high-resolution monochrome monitor (Audiotronics, North Hollywood, California).

Arteriolar diameter was continuously measured from the analog voltage output of the video analyzer, which had been modified to perform as a video micrometer. The system was calibrated with a stage micrometer to an accuracy of 1 μm.

Red blood cell velocity was continuously measured with a velocimeter (model 100, Optech Instruments, Durham, North Carolina) in series between the microscope and video camera. This instrument was calibrated by moving a clear plastic film coated with dried blood cells past the detector at known rates. The accuracy was <0.1 mm/sec.

Arterial blood pressure, arteriolar diameter, red blood cell velocity, and tissue temperature were continuously recorded on a dynograph. Blood flow was calculated off-line from the product of a constant, arteriolar diameter and red blood cell velocity.

Experimental Protocol

After a 30–40-minute postsurgery stabilization period, arterioles were selected for observation on the basis of size (20–60 μm), visual clarity, and briskness of red blood cell velocity. Sluggish blood flow indicated either partial vascular occlusion, surgical damage, or systemic hypotension and was cause for immediate rejection of the preparation. The level of spontaneous vasomotor tone was tested with the topical application of 10–3 M adenosine. The typical response was an increase in diameter and blood flow that was rapidly reversible on wash-out of adenosine. A preparation was rejected if it lacked vasomotor tone. Approximately 20% of the preparations were rejected because the viability or reactivity criteria were not satisfied.

Thereafter, ascending amounts of NECA (10–10–5×10–6 M), 2CA (10–10–10–4 M), CHA (10–10–10–6 M), or ADO (10–10–10–4 M) were added to the suffusate chamber. Diameter and blood flow data were collected after the suffusate had fully equilibrated with the tissue at each concentration (5–10 minutes). The substance was then removed from the suffusate, and measurements were continued until the arteriole stabilized at a posttreatment baseline. Less than 5% of the preparations were rejected because the pretreatment and posttreatment baselines were different.

In some experiments, 10–6–10–4 M 8-phenyl theophylline (8pTHEO), 10–7–10–4 M dipryridamole (DIPYRID), 10–5 M acetylcholine, or 10–4 M nor-epinephrine were added to the suffusate alone and in combination with the other agonists.

Statistical Analysis

Several agonists were applied in individual animals if the baseline was restored within 30 minutes after the first treatment. In all animals, the order of agonist application was randomized. All drugs were purchased from Sigma Chemical, St. Louis, Missouri. All values were expressed as
Receptors can cause the action of the antagonist 8pTHEO, these data suggest that stimulation of A1 and A2 receptors; the receptors could be located on nerve terminals, vascular tissue, or parenchymal cells. For details, see the text. NECA, N-ethyl carboxamidoadenosine; 2CA, 2-chloro adenosine; CHA, cyclohexyl adenosine.

Results

Figure 1 shows the diameter changes evoked by the topical application of synthetic agonists alone (panel A) and in the presence of 10^{-4} M dipyridamole (DIPYRID) (panel B) or 10^{-5} M 8-phenyl theophylline (8pTHEO) (panel C). All values are mean±SEM and all data represent steady-state responses. According to the nanomolar sensitivity of the vasconstrictor responses and the micromolar sensitivity of the vasodilator responses, the potency order of the synthetic agonists for the vascular responses, and the action of the antagonist 8pTHEO, these data suggest that stimulation of A1 and A2 receptors can cause vasoconstriction and vasodilation in the skin microcirculation. However, these observations do not suggest that skin microvessels are necessarily endowed with A1 and A2 receptors; the receptors could be located on nerve terminals, vascular tissue, or parenchymal cells. For details, see the text. NECA, N-ethyl carboxamidoadenosine; 2CA, 2-chloro adenosine; CHA, cyclohexyl adenosine.

Mean±SEM. Treatment effects were compared with analysis of variance and unpaired t tests at the 95% confidence interval.

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Mean±SEM. Treatment effects were compared with analysis of variance and unpaired t tests at the 95% confidence interval.
of effect of 10^{-5} M \text{8pTHEO} on CHA-induced vasodilation is not surprising because the selectivity of the synthetic agonist for extracellular A_1 receptors is lost at micromolar concentrations.\cite{5,6,12} Thus, the responses evoked by high concentrations of CHA should be cautiously interpreted.

Figure 2 shows that the responses evoked by low concentrations of ADO were essentially superimposed on those evoked by CHA; that is, ADO caused vasoconstriction below 10^{-8} M and vasodilation above 10^{-6} M with Ringer’s alone or Ringer’s+DIPYRID in the suffusate. The minimum value was 90±2% of control at 10^{-6} M. The ED_{50}s for vasoconstriction were 0.32 nM (diameter, 94±1% of control) for Ringer’s and 0.14 nM (diameter, 94±1% of control) for Ringer’s+DIPYRID (Table 1). ADO-induced vasoconstriction was completely antagonized by \text{8pTHEO}.

At higher concentrations, ADO evoked dose-related vasodilation (Figure 2); the ED_{50} for this response was 13 \mu M at a diameter of 130±3% of control. The dose-response curve to ADO with Ringer’s only in the suffusate was displaced to the right, compared with that of 2CA (Figure 1). However, when DIPYRID was added to the suffusate, the response evoked by ADO was potentiated; the ED_{50} was reduced to 2.7 \mu M at a diameter of 141±3% of control (Table 1). The dose-related vasodilation evoked by ADO+DIPYRID (Figure 2) was essentially superimposed on the responses evoked by 2CA alone or 2CA+DIPYRID (Figure 1 and Table 1). Since DIPYRID blocks cellular uptake of ADO, it is reasonable to conclude that cellular uptake reduces the extracellular ADO concentration and thereby attenuates the receptor-mediated action of exogenous ADO.

Whereas 10^{-5} M \text{8pTHEO} completely antagonized the vasoconstriction evoked by ADO and CHA in the nM range and attenuated the vasodilation evoked by NECA and 2CA in the low \mu M range (Figure 1 and Table 1), it did not alter the vasodilation evoked by ADO in the high \mu M range. This lack of effect is not surprising because \text{8pTHEO} is a competitive antagonist, and high concentrations of ADO simply overrode the receptor blockade. The maximum diameter increase evoked by 10^{-4} M ADO was 158±7% of control alone and 151±10% of control with ADO+10^{-5} M \text{8pTHEO} (Figure 2). These values are not significantly different. If the concentration of \text{8pTHEO} was raised to 10^{-4} M, then 10^{-4} M ADO caused a diameter increase that averaged 120±2% of control (n=6), which was significantly lower than the increase caused by 10^{-4} M ADO alone. However, these data should be regarded with caution because of the large baseline shift caused by 10^{-4} M \text{8pTHEO} (see above).

The changes in Figure 3 were qualitatively similar to those in Figures 1 and 2, which suggests that 20–60-\mu m arterioles evaluated in this study were important resistance vessels in the skin microcirculation.

Figure 3 shows the arteriolar blood flow changes evoked by the agonists alone (panel A) and in the presence (panel B) of the antagonist. In panel A, control blood flow averaged 3.6±0.9, 5.3±1.2, 3.9±0.8, and 2.9±0.5 nl/sec before NECA (n=7), 2CA (n=5), ADO (n=10), and CHA (n=9), respectively. With \text{8pTHEO} (panel B), the values were 2.6±0.7, 5.3±1.0, 3.2±1.0, and 3.3±0.9 nl/sec before NECA (n=9), 2CA (n=8), ADO (n=5), and CHA (n=7), respectively. There were no significant differences between or within the groups.

### Table 1. ED_{50} and Half-Maximal Diameter Responses

<table>
<thead>
<tr>
<th>Ringer’s only</th>
<th>10^{-5} M Dipyridamole</th>
<th>10^{-5} M 8-Phenyl theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED_{50} ((\times 10^{-6} \text{M}))</td>
<td>Half-maximal diameter ((% \text{ control}))</td>
</tr>
<tr>
<td>Dilatation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NECA</td>
<td>0.22±0.04 ((n=22))</td>
<td>134±3</td>
</tr>
<tr>
<td></td>
<td>1.3±0.3 ((n=27))</td>
<td>133±2</td>
</tr>
<tr>
<td>ADO</td>
<td>12.9±3.0 ((n=24))</td>
<td>130±3</td>
</tr>
<tr>
<td>Constriction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHA</td>
<td>0.007±0.005 ((n=30))</td>
<td>93±1</td>
</tr>
<tr>
<td>ADO</td>
<td>0.0003±0.0002 ((n=24))</td>
<td>94±1</td>
</tr>
</tbody>
</table>

NECA, N-ethyl carboxamido adenosine; 2CA, 2-chloro adenosine; ADO, adenosine; CHA, cyclohexyl adenosine.
NECA evoked dose-related increases in blood flow; the maximum was 451 ± 30% of control at 5 × 10⁻⁶ M without 8pTHEO (panel A) and 255 ± 11% of control with 8pTHEO (panel B).

The dose-related increases caused by 2CA averaged 240 ± 25% of control at 5 × 10⁻⁶ M and reached 304 ± 31% of control at 10⁻⁵ M (Figure 3A) but, at the same concentration, were reduced to 183 ± 18% and 278 ± 27% of control by 10⁻⁵ M 8pTHEO (Figure 3B).

Blood flow decreased in a dose-related manner to 68 ± 5% of control with 10⁻⁸ M ADO. With 10⁻⁵ M 8pTHEO in the suffusate, this response was blocked. At concentrations higher than 10⁻⁶ M, ADO increased blood flow in a dose-related manner to 228 ± 27% of control at 10⁻⁴ M. The dose-related increases evoked by >10⁻⁶ M ADO were not antagonized by 10⁻³ M 8pTHEO.

The biphasic response caused by CHA reached a minimum of 78 ± 7% of control at 10⁻⁴ M (Figure 3A) and was completely blocked by 10⁻⁵ M 8pTHEO (Figure 3B). At 10⁻⁶ M, blood flow was 148 ± 16% of control; this increase was not attenuated by 10⁻³ M 8pTHEO.

In a separate experiment with 10⁻³ M 8pTHEO in the suffusate, blood flow averaged 3.2 ± 0.9 nl/sec (n = 5). When 10⁻⁶ M norepinephrine was added to the suffusate, blood flow decreased to 0.5 ± 0.1 nl/sec (17 ± 2% of control). Thus, the blood flow decreases caused by CHA and ADO were a small fraction of those evoked by norepinephrine. In addition, the effect of 8pTHEO on those responses could not be attributed to a nonspecific reduction in vessel reactivity.

In summary, the data in Figures 1–3 show that 1) CHA and ADO caused vasoconstriction and blood flow reductions in the nM range with a similar potency; 2) the vasoconstrictor responses were completely blocked by 8pTHEO and not altered by DIPYRID; 3) the potency order for evoking vasodilation and blood flow increases in the micromolar range was NECA > 2CA > ADO; 4) the vasodilation evoked by the synthetic agonists was attenuated by 8pTHEO and not altered by DIPYRID, but ADO-induced vasodilation was potentiated by DIPYRID.

The vascular responses caused by each agonist were highly reproducible. For example, repeat applications of the same concentration of ADO caused responses that differed by 1.3 ± 1.7% (n = 10) and repeat applications of CHA caused responses that differed by 1.4 ± 1.7% (n = 11). Furthermore, no data were included unless the baseline was restored after each agonist was washed out of the suffusate. Despite the consistency of repeat applications of the same agonist, the order of application of the various agonists influenced the magnitude of the CHA- or ADO-induced vasoconstriction.

Figure 4 shows responses in subsets of the data population in Figure 1. Panel A shows ADO responses before and after the application of CHA.
phenomenon would not have been detected if multiple dose treatments had not been applied to individual animals.

The baseline values were identical (38 ± 3 μm, n = 16, and 39 ± 4 μm, n = 8). Before CHA, ADO caused a dose-related vasosonstrictioan to a minimum of 92 ± 2% of control at 10^−9 M. After CHA, ADO caused dose-related vasosonstriction that was significantly enhanced; the minimum was 87 ± 4% of control at 10^−9 M. The difference between the two curves increased with concentration.

Figure 4 (panel B) shows CHA responses before and after the application of A1 agonists (NECA, 2CA, or ADO). The baseline values were identical (39 ± 3 μm, n = 13, and 37 ± 2 μm, n = 21). If CHA was applied first in a sequence, then the dose-related vasosonstriction reached a minimum of 83 ± 2% of control at 10^−8 M. If CHA was applied after one of the A2 agonists had been applied, then the vasosonstriction was significantly less; the minimum was 91 ± 2% of control at 10^−8 M.

Thus, pretreatment with CHA enhanced ADO-induced vasosonstriction. Conversely, pretreatment with A2 agonists attenuated CHA-induced vasosonstriction.

Figure 5 shows paired diameter responses to ADO and either NECA, 2CA, or CHA. Unlike the parent molecule, the synthetic agonists are poor substrates for cellular uptake, 5,6,12 Therefore, a comparison between the responses caused by ADO and the synthetic agonists will reflect the degree to which cellular uptake and subsequent metabolism interfere with the expression of ADO-mediated vascular responses. However, changes in arteriolar diameter might not always accurately reflect ADO receptor stimulation. For details, see the text.

From 10^−10 to 10^−6 M, there was virtually no difference between the vascular response evoked by ADO and that evoked by CHA (Figure 5). This similarity suggests that cellular uptake did not significantly interfere with expression of the A1-mediated vascular response of exogenous ADO.

In contrast, the difference between the ADO response and the 2CA response progressively increased from 10^−8 M, reaching a peak difference of 35 ± 10% (n = 6) at 10^−6 M, (Figure 5). At higher concentrations, the difference between 2CA and ADO progressively decreased; the differences were 28 ± 7% and 26 ± 4% at 5 × 10^−6 M and 10^−5 M and finally declined to 4 ± 2% at 10^−4 M. Since 2CA and ADO have equal affinities for A1 and A2 receptors, and since 2CA is a poor substrate for the nucleoside transporter at submicromolar concentrations, 5,6,12 these results suggest that cellular uptake of exogenous ADO interferes with the expression of the A2-mediated vascular response at concentrations below 10^−8 M.
The differences between the ADO and NECA responses were greater than the differences between the 2CA and ADO responses; the peak difference at $10^{-6}$ M was 55±6% (Figure 5). Since NECA has a greater affinity for $A_2$ receptors than 2CA,5,6 the relation between the two curves is further evidence suggesting that uptake and/or metabolism of ADO interferes with expression of the $A_2$-mediated responses.

**Discussion**

CHA and ADO caused vasoconstriction in the nanomolar range whereas NECA, 2CA, and ADO caused vasodilation in the micromolar range. Most of these responses were antagonized by 8pTHEO. These observations are consistent with the conclusion that stimulation of $A_1$ and $A_2$ receptors can cause vasoconstriction and vasodilation in the skin microcirculation. Since arteriolar blood flow changes (Figure 3) were qualitatively similar to the diameter changes (Figures 1 and 2), the 20–60 μm arterioles observed in this study are probably important resistance vessels in the skin microcirculation.

Another important conclusion from this study is that $A_2$-mediated vasoconstriction evoked by exogenous ADO is attenuated by cellular uptake, while $A_1$-mediated vasoconstriction evoked by exogenous ADO is probably not altered. This interpretation is supported by two major observations; first, the cellular uptake inhibitor, DIPYRID, potentiated ADO-induced vasoconstriction but had minimal effects on ADO-induced vasoconstriction (Figure 2) and had minimal effects on either vasodilator or vasoconstrictor responses evoked by the synthetic agonists (Figure 1). Thus, it is unlikely that the effect of DIPYRID could be attributed to a non-specific alteration in vascular reactivity; second, there were minimal differences between the responses evoked by ADO and CHA in the nanomolar range, but there were dose-related differences between ADO and either NECA and 2CA in the micromolar range (Figure 5).

Since ADO causes vasodilation in virtually every vascular bed by a mechanism coupled to $A_1$ receptors (e.g., see Fredholm and Sollevi6), it is not surprising that ADO, 2CA, and NECA caused vasodilation in the skin microcirculation. Perhaps the most interesting finding from this study is that nanomolar concentrations of exogenous ADO or CHA caused sustained vasoconstriction. Although the magnitude of the vasoconstriction was small compared with that evoked by norepinephrine, the responses were consistent and probably receptor-mediated because they were completely antagonized by 8pTHEO (Figures 1–3).

An important limitation is that pretreatment with CHA enhanced ADO-induced vasoconstriction and pretreatment with $A_2$ agonists attenuated CHA-induced vasoconstriction (Figure 4). The meaning of this interaction is unknown and was unmasked only because each animal was exposed to more than one agonist. Even though the dose-related diameter changes were highly reproducible and the baseline was restored after washout of each agonist (see "Results"), these responses might reflect a persistent effect of the synthetic compounds. The N$^+$-substituted adenosine analogues are potent agonists at $A_1$ receptors and generally have higher lipid solubilities than $A_2$-selective agonists, so CHA might have dissociated from receptors at a different rate than ADO, 2CA, and NECA dissociated.11,12 The rate of elimination is further complicated by nonspecific binding.11,12 However, the explanation for the data in Figure 4 is unknown.

Although $A_1$ receptors are abundant in nervous tissue,16 there are few, if any, reports suggesting the existence of $A_2$ receptors in the extrarenal vasculature. In the kidney, $A_1$ stimulation causes vasoconstriction and inhibition of renin secretion.13 However, the renal vasoconstriction caused by submicromolar concentrations of ADO is transient. In this present study, the vasoconstriction caused by submicromolar concentrations of ADO was sustained (Figure 1–3), albeit small compared with that caused by norepinephrine (see "Results"). In the intestine, ADO can cause vasoconstriction in some conditions, but this response is probably not mediated by $A_1$ receptors.11 In the heart, $A_1$ stimulation reduces excitation of the atrioventricular node,18 but ADO is a well-known coronary vasodilator by an $A_2$ mechanism.19,20 Thus, with the exception of the kidney, $A_1$ receptors have most often been associated with extravascular tissues.

The synthetic agonists used in this study are poor substrates for the nucleoside transporter.5,6,12 For this reason, additional information might be obtained by comparing responses evoked by ADO, which is avidly transported, and those evoked by the synthetic compounds. From $10^{-10}$ to $10^{-6}$ M, there was no significant difference between the vascular responses evoked by CHA and ADO (Figure 5). Since CHA is not transported and is an $A_2$-selective agonist at nanomolar concentrations, it is reasonable to conclude that cellular uptake probably does not compromise the $A_2$-mediated vasoconstriction evoked by exogenous ADO. This conclusion is supported by the lack of effect of DIPYRID on the vasoconstriction evoked by ADO (Figure 2). A corollary is that the affinity of the $A_1$ receptors for ADO is greater than the affinity of the transporter proteins for exogenous ADO.

At higher concentrations, uptake and metabolism of exogenous ADO appears to attenuate the receptor-mediated vascular responses. This interpretation is based on the logic that both 2CA and ADO are nonselective receptor agonists and both compounds have higher affinities for $A_1$ receptors than CHA.5,6,12 Since 2CA is a poor substrate for the nucleoside transporter at concentrations <$10^{-6}$ M, the difference between the 2CA and ADO responses should reflect the degree to which cellular uptake reduces the concentration of exogenous ADO at the site of

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The document contains several paragraphs discussing the effects of adenosine and adenosine analogues on vascular responses, particularly focusing on the role of receptor-mediated responses and the influence of cellular uptake on these responses. It highlights the differences in effects observed with different agonists, the importance of receptor selectivity, and the role of cell uptake in modifying these effects. The discussion also touches on the clinical implications, such as the use of adenosine in cardiovascular pharmacology, and the potential mechanisms underlying these responses.
action. Accordingly, Figure 5 shows a progressive increase in the difference between the ADO and 2CA responses from $10^{-8}$ to $10^{-6}$ M, which suggests that uptake of ADO reduces expression of the $A_2$-mediated vasodilation. This interpretation is also supported by the observation that DIPYRID potentiates the vasodilation caused by ADO (Figure 2), without altering that caused by NECA or 2CA and by the observation that the paired difference between ADO and 2CA was less than the difference between ADO and NECA. Since NECA is a more potent $A_2$ agonist than 2CA, the diameter differences between NECA and ADO should be greater than the differences between 2CA and ADO. As shown in Figure 5, this prediction was confirmed. There is ample biochemical evidence that the affinity of various enzymes involved in adenosine nucleotide metabolism in vitro would fall between the affinities of the nanomolar affinity range of $A_1$ receptors and the micromolar affinity range of $A_2$ receptors. Thus, in context with the CHA versus ADO curve (Figure 5), these data suggest that uptake and subsequent metabolism of ADO interferes with expression of the $A_2$ effect, but not the expression of the $A_1$ effect.

A major limitation of this study is that cellular events mediated by ADO-receptor stimulation were not measured. These responses include changes in cyclic AMP formation and/or modulation of phosphatidylinositol turnover and alterations in Ca²⁺ availability. It must be emphasized that ADO receptors have been operationally defined by the effects of the various synthetic analogues on adenylylcyclase (or other cellular events), rather than by the effects of these substances on arteriolar diameter. In this present study, it was assumed that changes in arteriolar diameter and blood flow would reflect cellular changes because previous studies have correlated ADO receptor stimulation, cyclic AMP concentration, and tissue blood flow changes. However, arteriolar diameter in the intact skin microcirculation is influenced by a host of factors other than ADO. Furthermore, arteriolar diameter changes do not always reflect total tissue blood flow changes. Therefore, these observations must be cautiously extrapolated to more carefully controlled conditions in vitro where ADO receptor stimulation can be unequivocally linked to specific cellular events.

The location of $A_1$ and $A_2$ receptors in the skin is unknown. ADO is a potent, and apparently ubiquitous, modulator of neurotransmitter release by pre-synaptic actions coupled to calcium flux. Furthermore, ADO reportedly has biphasic effects on acetylcholine and noradrenaline release from brain slices. Thus, it is possible that $A_1$ and $A_2$ receptors are located on nerve terminals in the skin microcirculation. Perhaps nanomolar concentrations of ADO and CHA caused cutaneous vasoconstriction by receptor-mediated inhibition of the release of an endogenous vasodilator (e.g., acetylcholine and bradykinin). Alternatively, ADO or CHA might have favored the release of an endogenous vasoconstrictor (e.g., noradrenaline). Consequently, it cannot be assumed that the $A_1$ receptors are localized on skin microvessels even though $A_1$ stimulation caused cutaneous vasoconstriction. Further experiments must determine the source of extracellular ADO and the localization of ADO receptors within the skin.

Sympathetic outflow is undoubtedly a major physiological vasoconstrictor in the skin. In addition, there is probably a direct or indirect influence of cholinergic innervation. In contrast, there is relatively little information on the possible role of nonadrenergic, non-cholinergic mechanisms, even though several cutaneous vascular responses are resistant to cholinergic and adrenergic antagonists. It is tempting to speculate about the possible role of purinergic nerves in the skin, especially since some cutaneous perivascular nerves in the skin structurally resemble the purinergic nerves in the gastrointestinal tract. Moreover, ADO and adenine nucleotides are released alone and coreleased with noradrenaline from some perivascular nerves. However, further experiments are needed to explore this idea.

In summary, pharmacological responses suggest the presence of $A_1$ and $A_2$ adenosine receptors in the skin microcirculation, but the function and precise location of these receptors is not known.

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**KEY WORDS** • theophylline • vascular smooth muscle • hamster
Pharmacological evidence for A1 and A2 adenosine receptors in the skin microcirculation.
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Circ Res. 1989;65:176-184
doi: 10.1161/01.RES.65.1.176

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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