Vasodilator responses to adenosine and hyperemia are mediated by A<sub>1</sub> and A<sub>2</sub> receptors in the cat vascular bed

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Bivalacqua, Trinity J., Hunter C. Champion, David G. Lambert, and Philip J. Kadowitz. Vasodilator responses to adenosine and hyperemia are mediated by A<sub>1</sub> and A<sub>2</sub> receptors in the cat vascular bed. Am J Physiol Regulatory Integrative Comp Physiol 282: R1696–R1709, 2002; 10.1152/ajpregu.00394.2001.—Hemodynamic responses to adenosine, the A<sub>1</sub> receptor agonists N<sup>6</sup>-cyclopentyladenosine (CPA) and adenosine amine congener (ADAC), and the A<sub>2</sub> receptor agonist 5'-[(N-cyclopropyl)-carboxamido]-adenosine (CPCA) were investigated in the hindquarter vascular bed of the cat under constant-flow conditions. Injections of adenosine, CPA, ADAC, CPCA, ATP, and adenosine 5'-O-(3-thiotriphosphate) (ATP<sub>S</sub>) into the perfusion circuit induced dose-related decreases in perfusion pressure. Vasodilator responses to the A<sub>1</sub> agonists were reduced by the A<sub>1</sub> receptor antagonists KW-3902 and CGS-15943, whereas responses to CPCA were reduced by the A<sub>2</sub> antagonist KF-17837. Vasodilator responses to adenosine were reduced by KW-3902, CGS-15943, and by KF-17837, suggesting a role for both A<sub>1</sub> and A<sub>2</sub> receptors.

Vasodilator responses to ATP and the nonhydrolyzable ATP analog ATP<sub>S</sub> were not attenuated by CGS-15943 or KF-17837. After treatment with the nitric oxide synthase inhibitor N<sup>ω</sup>-nitro-L-arginine methyl ester, the cyclooxygenase inhibitor sodium meclofenamate, or the ATP-dependent K<sup>+</sup> channel antagonists U-37883A or glibenclamide, responses to adenosine and ATP were not altered. Responses to adenosine, CPA, and CPCA were increased in duration by rolipram, a type 4 cAMP phosphodiesterase inhibitor, but were not altered by zaprinast, a type 5 cGMP phosphodiesterase inhibitor. When blood flow was interrupted for a 30-s period, the magnitude and duration of the reactive vasodilator response were reduced by A<sub>1</sub> and A<sub>2</sub> receptor antagonists.

These data suggest that vasodilator responses to adenosine and the A<sub>1</sub> and A<sub>2</sub> agonists studied are not dependent on the release of cyclooxygenase products, nitric oxide, or the opening of K<sup>+</sup><sub>ATP</sub> channels in the regional vascular bed of the cat. The present data suggest a role for cAMP in mediating responses to adenosine and suggest that vasodilator responses to adenosine and to reactive hyperemia are mediated in part by A<sub>1</sub> and A<sub>2</sub> receptors in the hindquarter vascular bed of the cat.

Regional vascular bed; KF-17837; CGS-15943; reactive vasodilation

ADENOSINE, AN ENDOGENOUS PURINE nucleoside formed from the dephosphorylation of cAMP by the ectoenzyme 5'-nucleotidase, mediates a variety of physiological responses in mammalian tissues (11, 13, 14). Based on molecular cloning studies (14), physiological responses to extracellular adenosine are mediated by four adenosine receptor subtypes (A<sub>1</sub>, A<sub>2a</sub>, A<sub>2b</sub>, and A<sub>3</sub>). The A<sub>1</sub> receptor is found in greatest number in the brain, spinal cord, testis, and adipose tissue and is coupled to several second messenger systems. In atrial and ventricular myocytes, A<sub>1</sub> receptor binding inhibits the activation of adenylyl cyclase and increases an inwardly rectifying K<sup>+</sup> current, leading to negative chronotropic and inotropic effects on the heart (4). A<sub>2a</sub> receptor mRNA has been found in highest concentration in the brain and thymus gland, whereas the message for the A<sub>2b</sub> receptor subtype has been found in human intestinal epithelium, and both receptor subtypes have been identified in human cultured aortic endothelial cells (19, 32, 36). Activation of the A<sub>2</sub> receptor results in stimulation of adenylyl cyclase and may also involve stimulation of nitric oxide (NO) formation and activation of ATP-dependent K<sup>+</sup> (K<sup>+</sup><sub>ATP</sub>) channels (1, 21). However, in isolated coronary arteries of the dog, inhibition of NO synthesis did not affect the dilator properties of adenosine (22). High levels of mRNA for the A<sub>3</sub> receptor subtype have been identified in human lung and liver, and there is evidence that activation of this receptor subtype results in inhibition of adenylyl cyclase and stimulation of phospholipase C (40).

Physiological responses elicited by adenosine are varied, and A<sub>2</sub> receptor activation produces vasodilation in most vascular beds in a variety of species (10, 24, 43). In the rat renal artery, A<sub>1</sub> receptor activation produces constriction, whereas A<sub>2</sub> receptor activation leads to vasodilation (8, 16, 18, 21, 37). Adenosine has been shown to have hypertensive activity in the pulmonary circulation in a number of species (5, 24, 32), whereas in the feline pulmonary vascular bed, responses to adenosine are tone dependent with a pressor response mediated by A<sub>1</sub> activation under low-tone conditions and vasodilation mediated by A<sub>2</sub> activation observed under elevated-tone conditions (10). The contribution of K<sup>+</sup><sub>ATP</sub> channels to ischemic vasodilation during reactive hyperemia has been well characterized.
In the feline hindquarter vascular bed, reactive hyperemia has been shown to be mediated in part by the opening of $K_{ATP}$ channels and the release of NO (28). However, little is known about the contribution of adenosine A1 and A2 receptors in the regulation of the peripheral vascular bed and on the reactive hyperemic response in the cat. Until recently, potent selective adenosine receptor antagonists have not been available to investigate the receptor subtypes involved in mediating responses to adenosine in physiological and pathophysiological conditions. It has been reported that KW-3902 and CGS-15943 are adenosine A1 receptor antagonists, whereas KF-17837 is a selective A2 receptor antagonist (16, 20, 38). Therefore, the present study was carried out to determine the receptor subtype and the mechanisms involved in mediating vasodilator responses to adenosine and reactive hyperemia in the hindquarter vascular bed of the cat.

MATERIALS AND METHODS

One hundred and seventeen adult mongrel cats of either sex, weighing 2.4–4.6 kg, were sedated with ketamine hydrochloride (10–15 mg/kg im) and were anesthetized with pentobarbital sodium (30 mg/kg iv). Supplemental doses of pentobarbital sodium were given as needed to maintain a uniform level of anesthesia. The trachea was cannulated, and the animals breathed spontaneously or were ventilated with a Harvard model 607 ventilator at a volume of 40–60 ml at 15–22 breaths/min. An external jugular vein was catheterized for intravenous administration of drugs, and a carotid artery was catheterized for the measurement of systemic arterial pressure. For constant-flow perfusion of the hindquarter vascular bed, a 3- to 4-cm segment of the distal abdominal aorta was exposed through a ventral midline incision and was cleared of surrounding connective tissue by blunt dissection. After administration of heparin sodium (1,500 U/kg iv), the aorta was ligated, and catheters were inserted proximal and distal to the ligature. Branches of the aorta distal to the origin of the external iliac arteries were ligated to restrict blood flow to the hindlimb. The hindquarter vascular bed was denervated by ligating and cutting the lumbar sympathetic chain ganglia between L1 and L4. Blood was withdrawn from the proximal catheter and pumped at a constant rate with a Sigmamotor model T-8 pump into the distal aortic catheter. For the reactive vasodilator experiments, blood flow to the hindquarter vascular bed was interrupted by stopping the perfusion pump for a 30-s period. When the pump was started and blood flow was restored, a reactive vasodilator response was observed (28). Perfusion pressure was monitored from a lateral tap in the perfusion circuit located between the pump and the distal aortic catheter. Hindquarter perfusion pressure and systemic arterial pressure were measured with Statham P23 transducers and were recorded on a Grass model 7 polygraph. Mean pressures were derived by electronic averaging, and the flow rate was determined by timed collection and recorded from 24 to 34 ml/min. The agonists used in these experiments were injected directly in the superior mesenteric arterial perfusion circuit distal to the pump in small volumes in a random sequence.

In the first set of experiments, hindquarter responses to injections in the perfusion circuit of the adenosine A1 receptor agonist N6-cyclopentyladenosine (CPA), the A2 receptor agonist 5‘-N-(N-cyclopentyl)carboxamido-adenosine (CPCA), adenosine, ATP, and adenosine 5‘-O-(3-thiotriphosphate) (ATP-S) were investigated under constant-flow conditions, with doses expressed on a nanomole basis to take into account differences in molecular weight. In the second set of experiments, the effects of selective adenosine A1 receptor antagonists CGS-15943 and KW-3902 and the A2 receptor antagonist KF-17837 on vasodilator responses were investigated, with agonist responses being compared before and after the administration of CGS-15943 (0.5 mg/kg iv), KW-3902 (2 mg/kg iv), and KF-17837 (2–3 mg/kg iv). The doses of CGS-15943, KW-3902, and KF-17837 were determined in pilot experiments. In the third set of experiments, the selectivity of the blockade induced by the A1 and A2 antagonists was investigated, and, in the fourth set of experiments, the mechanism by which adenosine produces vasodilation in the hindquarter vascular bed was investigated. To investigate the role of NO, Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME; 100 mg/kg iv) was administered, and responses to adenosine were evaluated beginning 20 min after completion of L-NAME administration. Vasodilator responses to ACh were compared before and after L-NAME to assess the degree of NO synthase inhibition. To investigate the role of vasodilator prostaglandins in mediating responses to adenosine, the cyclooxygenase inhibitor sodium mfolename was injected in a dose of 2.5 mg/kg iv over a 10-min period, and responses were evaluated beginning 20 min after completion of the injection. Vasodilator responses to arachidonic acid were compared before and after sodium mfolename to assess the degree of cyclooxygenase inhibition. The role of $K_{ATP}$ channel activation was investigated, and responses to adenosine were compared before and after administration of the $K_{ATP}$ channel antagonist U-37883A or glibenclamide (5 mg/kg iv). Responses to the $K_{ATP}$ channel opener levocromakalim were compared before and after administration of U-37883A or glibenclamide to assess the $K_{ATP}$ channel blockade. In the fifth set of experiments, the role of cAMP and cGMP in mediating responses to adenosine, CPA, and CPCA was evaluated. Rolipram, a type 4 cAMP

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phosphodiesterase inhibitor, was injected in a dose of 0.5 mg/kg iv, and zanaprist, a type 5 cGMP phosphodiesterase inhibitor, was injected in a dose of 1 mg/kg iv. Responses to adenosine, CPA, and CPC were compared before and beginning 20 min after administration of rosiglitazone or zanaprist. In the final set of experiments, the role of A1 and A2 receptors in mediating the reactive hyperemic response was studied under constant-flow conditions in the hindquarter vascular bed of the cat. The effect of a 30-s period of interruption of hindquarter blood flow induced by stopping the perfusion pump was assessed in terms of total area under the perfusion pressure curve over time, the duration of the reactive hyperemic response, and the percent decrease in hindquarter perfusion pressure (28). In this set of experiments, the effects of CGS-15943, KP-17837, the combination of CGS-15943 and KP-17837, and of the passage of time on the hindquarter reactive vasodilator response were investigated.

Preparation of drugs. ATP, ATPyS, adenosine, acetylcholine bromide, l-NAME (Sigma Chemical, St. Louis, MO), and albuterol sulfate (Scherer, Kenilworth, NJ) were dissolved in 0.9% NaCl. U-37883A (Upjohn, Kalamazoo, MI) was dissolved in 0.9% NaCl with sonication. Glibenclamide (Sigma) was dissolved in a 10% ethanol/saline solution at a concentration of 10 mg/ml and was diluted with 0.9% NaCl. Levcromakalim (SmithKline Beecham, Sussex, UK) was dissolved in 20% ethanol/saline solution at a concentration of 1 mg/ml and was diluted with 0.9% NaCl. Zanaprist (2-O-propoxyphenyl-8-azapurin-6-one; Rhone-Poulenc, Degenham, Essex, UK) was dissolved in 0.15 N NaOH in normal saline in a concentration of 3 mg/ml. Rolidiprim (SmithKline Beecham) was dissolved in 20% dimethyl sulfoxide (DMSO) and diluted with normal saline. CGS-15943 (RBI, Natick, MA) was dissolved in DMSO with sonication. KF-17837 and KW-3902 (provided by Dr. Fumio Suzuki, Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo, Schizuoka, Japan) were dissolved in propylene glycol. CPA, adenosine amine congener (ADAC), and CPC (RBI were dissolved in 1 N acetic acid and diluted with normal saline. The vehicles for these agents had no consistent effect on baseline vascular pressure or responses to the vasoactive agonists. The drug solutions were stored in dark bottles in a freezer, and working solutions prepared on a frequent basis were kept on crushed ice during an experiment.

The hemodynamic data are expressed in absolute units as means ± SE, except in experiments with l-NAME in which baseline tone was markedly increased, and responses are expressed as percent decrease to take into account changes in baseline perfusion pressure. In experiments carried out to determine the role of A1 and A2 receptors on the response to reactive hyperemia, the area under the curve was measured with a planimeter or using a Bruning model 4849 area graph grid. The data were analyzed using a one-way ANOVA with repeated measures and Scheffe’s F-test with a Bonferonni/Dun procedure or a paired t-test. A P value < 0.05 was used as the criterion for statistical significance.

RESULTS

Responses to purinergic agonists. Responses to purinergic agonists were investigated in the hindquarter vascular bed of the cat under constant-flow conditions, and dose-response curves are shown in Fig. 1. Injections of the purinergic agonists into the hindquarter perfusion circuit produced dose-related decreases in perfusion pressure (Fig. 1). When doses are expressed on a nanomole basis, adenosine and the A2 receptor agonist CPA were the most potent vasodilators, with dose-response curves 2 log units to the left of the curve for the A1 agonist CPA and 1 log unit to the left of the dose-response curves for ATP and the degradation-resistant ATP analog ATPyS (Fig. 1).

Role of A1 and A2 receptors. The role of A1 and A2 receptors in mediating vasodilator responses to adenosine was investigated, and these data are summarized in Figs. 2–4. Decreases in hindquarter perfusion pressure in response to adenosine were decreased significantly [51 ± 4 to 21 ± 4 mmHg (59% decrease) at the 30-μg dose] after administration of the A2 receptor antagonist KF-17837 (2–3 mg/kg iv; Fig. 2). Treatment with KF-17837 significantly attenuated vasodilator responses to the A2 receptor agonist CPA [47 ± 3 to 14 ± 2 mmHg (70% decrease) at the 1-μg dose] without altering responses to the A1 agonists CPA or ADAC or to ATP or ATPyS (Fig. 2). KF-17837 had no significant effect on vasodilator responses to ACh, levcromakalim, or albuterol (data not shown).

The role of A1 receptors in mediating hindquarter responses to adenosine was investigated, and, after administration of the A1 receptor antagonist CGS-15943 (0.5 mg/kg iv), vasodilator responses to adenosine were reduced significantly [55 ± 7 to 35 ± 4 mmHg (36% decrease) at the 30-μg dose; Fig. 3]. After administration of CGS-15943, vasodilator responses to the A1 agonists CPA and ADAC were reduced significantly [33 ± 3 to 14 ± 4 mmHg (58% decrease) at 30 μg for CPA and from 42 ± 5 to 19 ± 4 mmHg (55% decrease) for ADAC at the 30-μg dose] without altering responses to the A2 agonist CPA (Fig. 3). CGS-15943 had no significant effect on vasodilator responses to ATP and ATPyS (Fig. 3) or on responses to ACh, levcromakalim, or albuterol (data not shown). In an additional set of experiments, the role of A1 receptors...
in mediating hindquarter responses to adenosine, CPA, and CPCA was investigated using the selective A<sub>1</sub> receptor antagonist KW-3902 (2 mg/kg iv), and these data are summarized in Table 1. Vasodilator responses to adenosine and the A<sub>1</sub> receptor agonist CPA were significantly decreased after treatment with KW-3902, whereas vasodilator responses to the A<sub>2</sub> receptor agonist CPCA were not changed significantly (Table 1). Treatment with KW-3902 had no significant effect on vasodilator responses to ATP, ACh, or albuterol (data not shown).

The combined effect of CGS-15943 and KF-17837 on the vasodilator response to adenosine was assessed and, after administration of CGS-15943 (0.5 mg/kg iv), the vasodilator response to adenosine was reduced significantly (46% decrease); the administration of KF-17837 (2–3 mg/kg iv) to the same animals produced a

![Figure 2](image-url)

**Fig. 2.** Influence of the adenosine A<sub>2</sub> receptor antagonist KF-17837 (2–3 mg/kg iv) on vasodilator responses to adenosine, CPCA, CPA, adenosine amine congener (ADAC), ATP, and ATPγS in the hindquarter vascular bed. Responses to the purinergic agonists were compared beginning 10–20 min after administration of the A<sub>2</sub> receptor antagonist. *Response is significantly different from control.

![Figure 3](image-url)

**Fig. 3.** Influence of the A<sub>1</sub> receptor agonist CGS-15943 (0.5 mg/kg i.v.) on vasodilator responses to adenosine, ADAC, CPA, ATP, and ATPγS in the hindquarter vascular bed. Responses to the purinergic agonists were compared before and beginning 10–20 min after administration of the A<sub>1</sub> receptor antagonist. *Response is significantly different from control.
significantly greater (73%) decrease in the response to adenosine than did CGS-15943 alone (Fig. 4).

Influence of NO synthase and cyclooxygenase inhibitors and a K<sub>ATP</sub> channel antagonist. To determine if hindquarter vasodilator responses to adenosine are mediated or modulated by the release of NO, responses were compared before and after administration of the NO synthase inhibitor L-NAME, and these data are summarized in Fig. 5A. After administration of L-NAME in a dose of 100 mg/kg iv, vasodilator responses to adenosine were not reduced at a time when responses to ACh were significantly decreased (Fig. 5A). The NO synthase inhibitor did alter vasodilator responses to albuterol or levcromakalim (data not shown).

To ascertain if responses to adenosine are modulated by the release of vasodilator products in the cyclooxygenase pathway, responses were compared before and after administration of the cyclooxygenase inhibitor sodium meclofenamate in a dose of 2.5 mg/kg iv, and these data are summarized in Fig. 5B. After administration of sodium meclofenamate, responses to adenosine were not significantly different from control, whereas vasodilator responses to the prostaglandin precursor arachidonic acid were reduced significantly (Fig. 5B).

The role of K<sub>ATP</sub> channels in mediating the response to adenosine was investigated, and, after administration of the K<sub>ATP</sub> channel antagonist U-37883A or glibenclamide (5 mg/kg iv), vasodilator responses to adenosine were not significantly reduced at a time when vasodilator responses to the K<sub>ATP</sub> channel opener levcromakalim were reduced significantly (Fig. 6, A and B).

In another set of experiments in the mesenteric vascular bed of the cat under constant-flow conditions, vasodilator responses to adenosine were compared before and after administration of U-37883A (5 mg/kg iv) to determine if K<sub>ATP</sub> channel activation plays a role in mediating responses to adenosine in another regional vascular bed in the cat. Vasodilator responses to adenosine were not significantly reduced at a time when responses to the K<sub>ATP</sub> channel opener levcromakalim were decreased after administration of U-37883A in the mesenteric vascular bed (Fig. 6C).

Influence of rolipram and zaprinast. The role of changes in cAMP and cGMP levels in mediating responses to adenosine in the hindquarter vascular bed was assessed by investigating the effects of type 4 cAMP and type 5 cGMP phosphodiesterase inhibitors on the duration of the vasodilator response as measured by the recovery half-time (T<sub>1/2</sub>) of the response. The T<sub>1/2</sub> is defined as the time required for the pressure to return to 50% of the maximal decrease in perfusion pressure. The time course of the decrease in hindquarter perfusion pressure in response to adenosine (3 μg) and albuterol and the effects of rolipram are shown in Fig. 7. After administration of the type 4 phosphodiesterase inhibitor rolipram (0.5 mg/kg iv), the T<sub>1/2</sub> of the vasodilator responses to adenosine, CPA, CPCA, and albuterol was increased significantly, whereas the T<sub>1/2</sub> of the response to the NO donor 2-(N,N-diethylamino)diazenolate 2-oxide (DEA/NO) was not altered (Figs. 7 and 8). The T<sub>1/2</sub> (s) of the response to adenosine was increased from 15 ± 1 to 23 ± 1 s after treatment with rolipram, and the T<sub>1/2</sub> (s) of the response to the β-agonist albuterol was increased from 52 ± 7 to 220 ± 25 s after treatment with rolipram (Figs. 7 and 8). The time course of the decreases in hindquarter perfusion pressure in response to adenosine and DEA/NO and the effect of zaprinast are shown in Fig. 9. The T<sub>1/2</sub> (s) of the vasodilator response to adenosine (3 μg) was not significantly different after treatment with zaprinast (15 ± 2 to 14 ± 1 s; Figs. 9 and 10). The T<sub>1/2</sub> of the vasodilator response to DEA/NO (3 μg) was increased.

Table 1. Effect of the adenosine A<sub>1</sub> receptor antagonist KW-3902 on vasodilator responses to adenosine, CPA, and CPCA in the hindquarters vascular bed of the cat

<table>
<thead>
<tr>
<th>Adenosine, μg</th>
<th>Control</th>
<th>KW-3902 (2 mg/kg iv)</th>
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<tr>
<td>3</td>
<td>-22 ± 3</td>
<td>-11 ± 2*</td>
</tr>
<tr>
<td>10</td>
<td>-35 ± 5</td>
<td>-22 ± 4*</td>
</tr>
<tr>
<td>CPA (10 μg)</td>
<td>-24 ± 3</td>
<td>-15 ± 3*</td>
</tr>
<tr>
<td>CPCA, μg</td>
<td>-12 ± 3</td>
<td>-10 ± 2</td>
</tr>
<tr>
<td>0.1</td>
<td>-26 ± 4</td>
<td>-23 ± 4</td>
</tr>
<tr>
<td>0.3</td>
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Values are means ± SE; n = 5–6 experiments. CPA, N<sup>e</sup>-cyclopentyladenosine; CPCA, 5'-[N-(cyclopropyl)-carboxamido]-adenosine. *P < 0.05, response is significantly different from control.
from 71 ± 18 to 104 ± 16 s after treatment with zaprinast (Figs. 9 and 10). After administration of the type 5 cGMP phosphodiesterase inhibitor zaprinast (1 mg/kg iv), vasodilator responses to adenosine, CPA, CPCA, and albuterol were not altered, whereas the $T_{1/2}$ of the vasodilator response to DEA/NO was increased significantly (Figs. 9 and 10).

Role of A1 and A2 receptors in the reactive hyperemic response. The effects of CGS-15943 (0.5 mg/kg iv) and of KF-17837 (3 mg/kg iv) on the reactive vasodilator response to a brief period of ischemia were investigated; these data are summarized in Fig. 11. When blood flow was restored after a 30-s period of ischemia, a significant decrease in hindquarter perfusion pressure (vasodilator response) lasting $125 \pm 5$ s was observed (Fig. 11). The hyperemic response to a 30-s period of arterial inflow occlusion was reduced after administration of CGS-15943, KF-17837, and the combination of CGS-15943 and KF-17837 (Fig. 11, A-C). The area under the curve, the duration of the reactive vasodilation, and the percent decrease in hindquarter perfusion pressure after a 30-s period of arterial occlusion were not reduced after treatment with vehicle (Fig. 11D).

Effects on baseline tone. The effects of the antagonists and inhibitors used in the present study on mean systemic arterial and hindquarter perfusion pressures are summarized in Table 2. Because cardiac output was not measured, changes in total peripheral resistance could not be analyzed. However, because blood flow to the hindquarter vascular bed was maintained constant, changes in perfusion pressure reflect changes in vascular resistance in the bed. The largest increase in vascular resistance observed was in experiments with L-NAME, suggesting that NO release plays an important role in regulating vascular tone. A large increase in baseline tone, by changing initial value, will enhance vasodilator responses; therefore, in experiments with the NO synthesis inhibitor, vasodilator responses are expressed in terms of the percent decrease in perfusion pressure to take changes in initial tone into account. In these experiments, both negative
(albuterol and levromakalim) and positive control agonist responses (ACh) are used to assess changes in vascular responsiveness. The cyclooxygenase inhibitor sodium meclofenamate and the KATP/H11001 channel antagonists had only small effects on perfusion pressure, and the efficacy of the pharmacological probes was assessed using the prostaglandin precursor arachidonic acid and the KATP channel opener levromakalim.

The phosphodiesterase inhibitors rolipram and zaprinast caused significant decreases in hindquarter vascular resistance, suggesting a role for cAMP and cGMP turnover in regulating baseline tone in this bed. The effects of these agents on response duration, as shown in Figs. 7–10, were evaluated using appropriate negative and positive control agonist injections to assess changes on response duration.

Both KF-17837 and CGS-15943 caused small significant increases in perfusion pressure, suggesting a role for tonic activation of adenosine receptors in the regulation of baseline tone in the vascular bed. The efficacy and selectivity of the blockade were assessed using selective A1 and A2 receptor agonists. Neither KF-17837 nor CGS-15943 altered vasodilator responses to ACh, albuterol, or levromakalim, and KW-3902 did not alter responses to ATP, ACh, albuterol, or levromakalim. In addition to assessing the selectivity and efficacy of the inhibitory effects of the pharmacological probes used, control experiments were carried out to
assess the effects of time and the vehicles used in this study on vascular responses.

**DISCUSSION**

New findings from this study are that vasodilator responses to adenosine in the hindquarter vascular bed of the cat are mediated by A1 and A2 receptors, whereas the release of NO, vasodilator prostaglandins, and the opening of KATP channels appear to have no important role. The present data suggest a role for cAMP in mediating vasodilator responses, and, in addition, these results show that A1 and A2 receptor antagonists reduce the reactive hyperemic response after a 30-s period of ischemia in the hindquarter vascular bed of the cat.

The results of the present investigation show that adenosine, the A1 receptor agonists CPA and ADAC, and the A2 receptor agonist CPCA produce dose-related decreases in hindquarter perfusion pressure in the cat (Fig. 1). Inasmuch as blood flow to the hindquarter vascular bed was maintained constant, the decreases in perfusion pressure reflect decreases in hindquarter vascular resistance. In terms of relative vasodilator activity, adenosine and CPCA were approximately equivalent and were about 100-fold more potent than the A1 receptor agonist CPA. ATP and the
degradation-resistant ATP analog ATPγS have significant vasodilator activity and were halfway between the A₁ and A₂ receptor agonists in vasodilator potency (Fig. 1). The results of experiments with the A₁ and A₂ receptor agonists suggest that A₁ and A₂ receptors mediating vasodilation are present in the hindquarter vascular bed of the cat (Figs. 1–3). To further test the hypothesis that A₁ and A₂ receptors are present and to determine the role of these receptor subtypes in mediating the response to adenosine, the effects of the A₂ receptor antagonist KF-17837 and the A₁ antagonists CGS-15943 and KW-3902 were investigated. KF-17837 attenuated responses to the A₂ agonist CPCA without altering responses to the A₁ agonists CPA and ADAC, and the A₂ receptor antagonist reduced the vasodilator response to adenosine (Fig. 2). These data indicate that A₂ receptors mediating vasodilation are present and that vasodilator responses to adenosine are mediated in part by the activation of A₂ receptors, a finding consistent with results from a number of studies (11, 14, 23, 43). The A₁ receptor antagonists CGS-15943 and KW-3902 decreased vasodilator responses to the A₁ agonist CPA without altering responses to the A₂ agonist CPCA (Fig. 3 and Table 1). These results provide support for the hypothesis that A₁ receptors mediating in part the vasodilator response to adenosine are present in the hindquarter vascular bed. This finding is at variance with results from a number of studies.
indicating that A2 receptors mediate vasorelaxant responses to adenosine and that A1 receptors have no significant role (11, 18, 43). Earlier studies have shown that the hypotensive response to CPA may be the result of bradycardia and a reduction in cardiac output after A1 receptor activation (34). However, in the present study under constant-flow conditions, vasodilator responses to direct local injections of the A1 receptor agonists CPA and ADAC into the perfusion circuit were rapid in onset, suggesting that responses to CPA and ADAC were the result of a direct effect on A1 receptors on resistance elements in the hindquarter vascular bed. These results are consistent with the results of studies in the hindlimb and diaphragmatic vascular beds in the rat (6, 12). The observation that higher doses of the A1 agonist relative to doses of the A2 agonist were required to induce vasodilation suggests that the A1 receptor mediating vasodilation in the hindquarter vascular bed of the cat may be of an unusual low-affinity type and is in agreement with a previous study in which high concentrations of CPA

![](Fig. 11. Bar graphs showing the effect of CGS-15943 (0.5 mg/kg iv; A), KF-17837 (3 mg/kg iv; B), CGS-15943 (0.5 mg/kg iv) and KF-17837 (3 mg/kg iv; C), and saline (D) on the area under the curve (AUC, in mmHg-min), the duration of the reactive vasodilator response, and the percent decrease in hindquarter perfusion pressure in response to a 30-s period of arterial inflow occlusion. The reactive hyperemic responses were compared before and beginning 10 min after injection of CGS-15943 or KF-17837 and after administration of CGS-15943 and KF-17837 or saline. n, No. of experiments. *Response is significantly different from control.

Table 2. Effect of antagonists and inhibitors used in this study on systemic arterial pressure and hindquarter perfusion pressure in the cat

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<thead>
<tr>
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<th>Systemic Arterial Pressure</th>
<th>Hindquarter Perfusion Pressure</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>101 ± 8</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>108 ± 7</td>
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<tr>
<td>KF-17837 (2–3 mg/kg iv)</td>
<td>10</td>
<td>104 ± 7</td>
</tr>
<tr>
<td>CGS-15943 (0.5 mg/kg iv)</td>
<td>7</td>
<td>128 ± 9*</td>
</tr>
<tr>
<td>L-NAME (100 mg/kg iv)</td>
<td>8</td>
<td>83 ± 11</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>108 ± 10</td>
</tr>
<tr>
<td>Meclofenamate (2.5 mg/kg iv)</td>
<td>7</td>
<td>119 ± 8</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>105 ± 5</td>
</tr>
<tr>
<td>U-37883A (5 mg/kg iv)</td>
<td>10</td>
<td>111 ± 6</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>101 ± 11</td>
</tr>
<tr>
<td>Rolipram (0.5 mg/kg iv)</td>
<td>8</td>
<td>93 ± 7*</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>72 ± 8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. L-NAME, Nω-nitro-l-arginine methyl ester. *P < 0.05, pressure is significantly different from control.
were required to relax isolated bovine coronary arterial rings (27).

Another possible explanation for the vasodilation observed in response to injections of the A₁ receptor agonists may be that CPA and ADAC are interacting with A₂ receptors. However, the high selectivity of CPA (26) and ADAC (44) for the A₁ receptor and the absence of an inhibitory effect of the A₃ antagonist KP-17837 on responses to CPA and ADAC suggest that activation of the A₂ receptor would not account for the observed results. Furthermore, the A₁ receptor antagonist KU-3902 also attenuated vasodilator responses to CPA at a time when responses to CPCA were not altered in the hindquarter vascular bed of the cat (Table 1; see Refs. 10 and 34). These results suggest that A₂ receptor activation does not contribute to the vasodilator response to CPA or ADAC in the present study. Previous reports have shown that CGS-15943 acts as a nonselective A₁ and A₂ receptor antagonist; however, the present data indicate that CGS-15943 in the dose used is selective for A₁ receptors in the hindquarter vascular bed of the cat (16, 44). The selectivity of an antagonist for a receptor could depend on the species, experimental preparation, or vascular bed studied, and, in pilot studies, high doses of CGS-15943 also attenuated responses to the A₂ agonist CPA. The results of the present study showing that vasodilator responses to ATP and ATP_{γS} are similar, that these agents are less potent than adenosine, and that responses are not altered by A₁ or A₂ receptor antagonists suggest that ATP breakdown and adenosine formation do not contribute to vasodilator responses to ATP in the hindquarter vascular bed and are in agreement with studies in the pulmonary vascular bed in this species (10, 32).

It has been reported that vasorelaxant responses to adenosine are endothelium dependent, involving the release of NO, and are dependent on the release of cyclooxygenase products or the opening of K⁺ATP channels (1, 20, 23, 43). The mechanism underlying vasodilator responses to adenosine was investigated, and responses to the purinergic agonist were not altered after administration of the NO synthase inhibitor L-NAME or the cyclooxygenase inhibitor sodium miconidate in doses that decreased responses to ACh and arachidonic acid (Fig. 5). These data suggest that adenosine does not induce vasodilation by releasing NO or products in the cyclooxygenase pathway in the hindquarter vascular bed. The role of K⁺ATP channel activation was investigated in the hindquarter and mesenteric vascular beds of the cat, and, after treatment with the K⁺ATP channel antagonist U-37883A or glibenclamide in doses that reduced vasodilator responses to the K⁺ATP channel opener levcromakalim, responses to adenosine were not altered (Fig. 6), suggesting that the opening of K⁺ATP channels is not involved in mediating the vasodilator response to adenosine in the hindquarter and mesenteric vascular beds of this species.

Adenosine A₂ receptors are reported to be coupled to adenylyl cyclase and increase cAMP levels (23); in studies on the mechanism by which adenosine dilates the hindquarter vascular bed, the duration of the vasodilator response before and after administration of the type 4 cAMP and type 5 cGMP phosphodiesterase inhibitors was measured. After treatment with the type 4 inhibitor rolipram in a dose that significantly increased the duration of the response to the B₂ receptor agonist albuterol, the T_{1/2} of the vasodilator response to adenosine, CPA, and CPCA was increased significantly, whereas the duration of the response to DEA/NO was not altered (Figs. 7 and 8). There are numerous reports in the literature that associate the A₁ receptor with inhibition, not stimulation, of adenylyl cyclase (13, 38, 43). However, in the hindquarter vascular bed of the cat, A₁ receptor activation does not appear to decrease cAMP formation, which could evoke a vasoconstrictor response. The type 5 cGMP phosphodiesterase inhibitor zaprinast, in a dose that increased the duration of the vasodilator response to DEA/NO, did not alter the T_{1/2} of the response to adenosine, CPA, CPCA, or albuterol (Figs. 9 and 10). These data suggest that vasodilator responses to adenosine, CPA, and CPCA are not associated with an increase in cGMP levels in the hindquarter vascular bed of the cat and that increases in cAMP levels not mediated by the release of a vasodilator prostaglandin may be involved.

The signal transduction mechanism for A₁ receptors has been extensively studied, and the original definition of A₁ receptor activation was based on inhibition of adenylyl cyclase (17, 20). The inference that A₁ receptor activation may be associated with increased cAMP formation goes against a great deal of biochemical evidence in the literature (17, 20, 23, 43). A decrease in cAMP levels in vascular smooth muscle is usually associated with vascular smooth muscle contraction and vasoconstriction. The results of the present study and previous results in the literature show that A₁ receptor activation results in vasodilation (6, 7, 12). The present data differ from data in several studies in which A₁ receptor-induced vasodilation has been associated with the activation of K⁺ATP channels or the release of NO (1, 20, 23, 25, 43). Although there is disagreement about the role of K⁺ATP channels or NO in A₁ receptor-mediated responses, the present study is the only known data suggesting that vasodilator responses to A₁ activation may be associated with increased cAMP levels in resistance vessel elements in the hindlimb circulation of the cat. We therefore wish to be very cautious in suggesting a relationship between A₁ receptor activation and increased cAMP levels. The doses of the A₁ agonists CPA and ADAC required to induce vasodilation were much higher than doses of the A₂ agonist CPA or adenosine. It is known in isolated tissue studies that the affinity of CPA or ADAC for the A₁ receptor is as high as CPA for the A₂ receptor (11, 27). Moreover, neither CPA nor ADAC is specific and, at high concentrations, can activate A₁ receptors. The present data are unusual in that the A₁ receptor, which in most studies mediates a contractile response, appears to be of the low-affinity type (11, 27). In the present study, both CPA and ADAC are full agonists capable of inducing a maximal vasodilator
response at high doses, and responses to CPA and ADAC are not blocked by KF-17837 in doses that block responses to CPCA. Moreover, responses to CPA and ADAC are blocked by CGS-15943 in a dose that does not block the response to CPCA. In experiments with the selective A1 antagonist KW-3501, responses to CPA were decreased significantly at a time responses to CPCA are not changed.

In regard to the question of receptor affinity, results of experiments in isolated porcine coronary arterial rings show that CPA causes contraction at low concentrations (10⁻⁸ to 10⁻⁶ M) and relaxation at high concentrations (10⁻⁶ to 10⁻⁵ M), whereas the A2 agonist N⁶-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]-adenosine (DPMA) caused relaxation at low concentrations (27). The results of this study in isolated coronary arterial rings are similar to the present data in that high concentrations of the A1 agonist CPA were required to induce vasorelaxation. It is therefore postulated that the A1 receptor mediating vasodilation in some organ systems may be an usual low-affinity-type receptor.

Reactive hyperemia is the increase in blood flow observed after a period of arterial occlusion and has been described in a number of vascular beds from a variety of species (2–5, 8, 28, 35, 39, 42). In the rat mesenteric circulation, adenosine receptor antagonists reduced the reactive hyperemic response (35). Furthermore, the coronary reactive hyperemic response in the dog was decreased after treatment with adenosine deaminase, suggesting that adenosine mediates the increase in blood flow after a brief period of ischemia (39). These results suggest that adenosine accounts for approximately one-third of the blood flow increase after a period of ischemia and suggest that other vasoactive factors play a role in mediating the hyperemic response. In the present study under constant-flow conditions in the hindquarter vascular bed of the cat, the A1 receptor antagonist CGS-15943 and the A2 receptor antagonist KF-17837 attenuated the maximal observed decrease in hindquarter vascular resistance, the duration of the reactive hyperemic response, and the area under the curve after a 30-s period of ischemia (Fig. 11). The reactive hyperemic response was reproducible with respect to time and was not dependent on the presence of an intact sympathetic innervation. The reduction in the response to a 30-s period of ischemia after treatment with A1 and A2 receptor antagonists suggests that the reactive hyperemic response in the hindlimb vascular bed is mediated in part by the activation of adenosine receptors (Fig. 11). The inhibitory effect of treatment with both CGS-15943 and KF-17837 on the duration of the response to a 30-s period of ischemia and the percent decrease in hindquarter perfusion pressure were not significantly greater than the inhibitory effect of CGS-15943 or KF-17837 alone. The reason that the inhibitory effect of combined treatment is not significantly greater than treatment with CGS-15943 or KF-17837 alone is uncertain but may be related to the complex nature of the reactive hyperemic response. In previous studies in the hindlimb vascular bed of the cat, the reactive hyperemic response was in part attenuated by K⁺ₐₜₚ channels and the release of NO may all play a role in mediating the reactive hyperemic response. The results of the present investigation and of previous studies in the literature provide support for the hypothesis that the reactive hyperemic response in several vascular beds in different species may in part involve the activation of adenosine receptors (35, 39).

In summary, the results of the present investigation suggest that A1 and A2 receptors mediating vasodilation are present in the hindquarter vascular bed of the cat and that adenosine acts on both receptor subtypes to induce vasodilation. Moreover, the present data suggest that vasodilator responses to ATP are not mediated by adenosine formed from ATP breakdown and that the response to adenosine is increased in duration by a cAMP phosphodiesterase inhibitor but is not altered by inhibitors of NO synthase or cGMP phosphodiesterase. Furthermore, vasodilator responses to adenosine are not altered by inhibitors of K⁺ₐₜₚ channel activation or the cyclooxygenase pathway. In addition, the reactive hyperemic response after a brief period of arterial occlusion is dependent in part on the activation of adenosine receptors. These data suggest that vasodilator responses to adenosine and the reactive hyperemic response are mediated at least in part by the activation of A1 and A2 receptors. These results also suggest that responses to adenosine do not involve the release of NO, vasodilator prostaglandins, or the opening of K⁺ₐₜₚ channels in the hindquarter vascular bed of the cat and suggest that increases in cAMP levels may be involved.

**Perspectives**

The broad implications of the present study are that, since vasodilator responses to adenosine are reduced by A1 and A2 selective receptor antagonists, these results suggest that both receptor subtypes are present and mediate responses to adenosine. The observation that responses to adenosine are increased in duration by a cAMP phosphodiesterase inhibitor but are not altered by inhibitors of K⁺ₐₜₚ channels, NO synthase, or cyclooxygenase suggests that the response is mediated in part by an increase in cAMP levels but that K⁺ₐₜₚ channel opening, the release of NO, or vasodilator prostaglandins are not involved. The observation that the response to reactive hyperemia is reduced by A1 and A2 receptor antagonists suggests that the release of adenosine from an endogenous source mediates in part the reactive hyperemic response by activating A1 and A2 receptors in the hindlimb circulation.

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REFERENCES


