Angiotensin Subtype 1 Blockade Selectively Potentiates Adenosine Subtype 2–Mediated Vasodilation

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A previous report demonstrated that infusion of adenosine into the forearm increased local vascular production of angiotensin II. We hypothesize that this increase in angiotensin II could attenuate the vasodilator response to adenosine subtype 2 (A₂) receptor activation. The depressor and regional hemodynamic responses to the A₂-selective adenosine agonist DPMA were measured in the presence and absence of angiotensin subtype 1 (AT₁) receptor blockade (losartan, 10 mg/kg IV) in anesthetized rats. Losartan pretreatment (without versus with losartan) significantly potentiated DPMA-induced reductions in renal (–13±2% versus –22±4%, P<.05) and mesenteric (–11±2% versus –23±4%, P<.05) vascular resistances, resulting in a greater depressor response (–7±2 versus –18±3 mm Hg, P<.05). The decrease in hindquarter vascular resistance was not affected. To test the specificity of this interaction, we also evaluated nitroglycerin and nifedipine. Pretreatment with losartan had no effect on the responses to nitroglycerin, whereas the responses to nifedipine either were not affected or were attenuated (percent change in mesenteric vascular resistance: without losartan pretreatment, –30±1%; with losartan pretreatment, –24±2%, P<.05). To determine whether the decrease in arterial pressure after losartan pretreatment contributed to the potentiation of the DPMA-mediated effects, we infused nitroglycerin to lower mean arterial pressure comparably to losartan treatment. None of the hemodynamic responses to subsequent DPMA administration were affected. These data suggest that endogenous levels of angiotensin II, whether released locally or systemically, selectively attenuate the A₂-mediated reductions in renal and mesenteric vascular resistances. (Hypertension 1993;22:221-230)

Key Words • hemodynamics • angiotensin II • adenosine • nifedipine • nitroglycerin

A great deal of evidence has accumulated indicating the existence of a vascular renin-angiotensin system and that angiotensin II (Ang II) can be formed within the vascular wall. Infusion of the β-adrenergic receptor agonist isoproterenol into the forearm has been shown to increase the local production of Ang II, presumably mediated by an increase in intracellular cyclic adenosine 3',5'-monophosphate (cAMP). Recently, the same group of investigators also demonstrated that infusion of adenosine increased the local production of Ang II.

Currently, adenosine receptors are classified into two subtypes, A₁ and A₂. In general, A₁ receptors are negatively coupled to adenylate cyclase and are linked to K⁺ channels, whereas A₂ receptor activation is associated with an increase in cAMP. The ability of adenosine to increase local angiotensin formation is most likely mediated by A₂ receptor activation, since Taddei et al have shown that agents which increase cAMP can stimulate local Ang II formation. The concept of A₂ receptors being responsible for local angiotensin release is supported by the demonstration that A₁ receptor activation decreases whereas A₂ receptor activation increases renal renin release.

Intravenous administration of A₂-selective agents has been shown to dose-dependently reduce mean arterial pressure and vascular resistance in the renal, mesenteric, and hindquarters circulations in the rat. The hypothesis tested in the present experiments was that adenosine-stimulated local production of Ang II would offset the A₂-mediated reductions in vascular resistance. We chose the A₂-selective adenosine agonist DPMA (N6-[2-(3,5-dimethoxyphenyl)-2-(2-methyl phenyl)ethyl]adenosine), which is 13-fold selective for A₂ over A₁, for use in these studies. The effects of angiotensin on the DPMA-induced vasodilations were assessed by blockade of the angiotensin type 1 (AT₁) receptors with the nonpeptidic antagonist losartan (DuP 753). Mean arterial pressure, heart rate, and regional hemodynamic responses to DPMA with and without AT₁ blockade were determined. Two mechanistically distinct vasodilators, nifedipine and nitroglycerin, were also evaluated with and without AT₁ blockade to determine if the potentiation of DPMA-mediated vasodilation by losartan is a generalized phenomenon associated with other vasodilators. In addition, the hemodynamic responses to DPMA with and without nitroglycerin infusion were measured to determine if the potentiation of DPMA by losartan pretreatment was due to changes in baseline arterial pressure or was specific for blockade of AT₁ receptors.
Methods

General Preparation

All experiments were conducted in accordance with a protocol approved by the Rhône-Poulenc Rorer Animal Care and Use Committee and conform to the National Institutes of Health Guidelines for the Use and Care of Laboratory Animals. Male Sprague-Dawley rats (Harlan Sprague Dawley, Inc, Indianapolis, Ind) weighing 300 to 400 g were anesthetized with thiobutabarbitral (100 mg/kg IP; BYK-Gildan, Constance, FRG), and the trachea was cannulated to ensure airway patency. A polyethylene catheter (PE50) was inserted into a femoral artery for measurement of arterial pressure. For intravenous administration of compounds, polyethylene catheters (PE50) were inserted into the right external jugular and femoral veins. As previously described, miniature pulsed-Doppler flow probes were placed on the right renal artery, superior mesenteric artery, and abdominal aorta for measurement of blood flow to the kidney, mesentery, and hindquarters, respectively. All incisions were closed with 8-mm stainless-steel wound clips, and the animal was placed on a servo-controlled heating pad. Thirty minutes was allowed after completion of surgery for full stabilization of the preparation.

Arterial pressure was measured by connecting the arterial catheter to a TNF-R transducer (Spectramed Inc, Oxnard, Calif) and was recorded on a polygraph (model 7, Grass Instrument Co, Quincy, Mass). Blood flows were measured with a model 545-C directional pulsed-Doppler flowmeter (Biengineering, University of Iowa, Iowa City) connected to the polygraph. The analog output from the polygraph was fed into a DAS-16 A/D board (Metabyte, Taunton, Mass) within a 386 personal computer. Digital data were collected by the CHAD data acquisition program (Rutland Inc, Clay- ton, Mo) in 3-second durations every 5 seconds. All values (mean arterial pressure, heart rate, and regional hemodynamics) were taken at the maximal depressor response at each dose of the compounds described below.

Intravenous Dose-Response of DPMA With and Without Losartan Pretreatment

The hemodynamic responses (mean arterial pressure, heart rate, and regional blood flows) to cumulative doses of the A1-selective adenosine agonist DPMA (synthesized by Rhône-Poulenc Rorer Department of Medicinal Chemistry) were determined (n=6). The compound was initially dissolved in dimethyl sulfoxide (DMSO) and diluted to a final concentration of 2 mg/mL with a 50:50 solution of polyethylene glycol (PEG200) and saline (solvent: 5% DMSO, 47.5% PEG200, and 47.5% of 0.9% NaCl). This solution was serially diluted to 1, 2, 7, and 20 μg/mL with saline. The animals were dosed intravenously with vehicle (corresponding to the DMSO/PEG200/saline concentrations in the 20 μg/mL solution) and 1, 3, 10, and 30 μg/kg DPMA in a cumulative dosing regimen. Following the peak effect after 30 μg/kg DPMA, as determined by the maximum reduction in mean arterial pressure, the adenosine receptor antagonist CGS 15943 (CIBA-GEIGY Pharmaceuticals, Summit, NJ) was administered at 250 μg/kg IV to determine if the responses to DPMA were mediated by adenosine receptor activation. CGS 15943 was dissolved in PEG200 at 2.5 mg/mL.

In a separate set of rats (n=6), the intravenous dosing of DPMA was repeated. However, this group received a single dose of losartan at 10 mg/kg IV 15 minutes before DPMA dosing. This dose of losartan was determined to be supramaximal and of adequate duration to ensure complete functional blockade of AT1 receptors throughout the time of the DPMA dosing. In a separate series of experiments (n=5), the ED99 of losartan (the dose...
DPMA

that completely blocked the pressor response to an infusion of 100 ng/kg per minute IV Ang II) was determined to be 6±2 mg/kg IV. The duration of action of losartan was determined in an additional set of animals (n=5). Intravenous infusion of 100 ng/kg per minute Ang II increased arterial pressure from 111±7 to 153±3 mm Hg. Administration of 10 mg/kg IV losartan returned arterial pressure to control. Arterial pressure was 117±8, 113±8, and 106±9 mm Hg at 10, 30, and 60 minutes after dosing with losartan, respectively. The responses to DPMA in the losartan-pre-treated animals were compared with the responses to DPMA in nonpretreated rats.

Intravenous Dose-Response of Nifedipine With and Without Losartan Pretreatment

To demonstrate that the interactions observed with DPMA and losartan pretreatment were selective, ie, a vasodilator with a different mechanism of action would not display the same interaction, we determined the hemodynamic responses (mean arterial pressure, heart rate, and regional blood flows) to cumulative doses of the calcium channel blocker nifedipine in two groups of rats (n=6, both groups). One group was pretreated with losartan, and the other was not.

Nifedipine (Sigma Chemical Co, St Louis, Mo) was initially dissolved in 5% DMSO, 47.5% PEG200, and 47.5% saline to 2 mg/mL and serially diluted to 3, 7, 20, and 70 μg/mL with saline. The animals were dosed with vehicle (corresponding to the concentrations of DMSO/PEG200/saline in the 70 μg/mL solution) and 3, 10, 30, and 100 μg/kg of nifedipine in a cumulative dosing regimen. As stated above, one group of rats received a 10 mg/kg IV pretreatment of losartan 15 minutes before nifedipine dosing, and the other group was not dosed with losartan. The responses to nifedipine in the losartan-treated animals were compared with the responses to nifedipine in nonpretreated rats.

Intravenous Dose-Response of Nitroglycerin With and Without Losartan Pretreatment

To evaluate further the specificity of the interaction between DPMA and losartan, we determined the hemodynamic responses to increasing doses of the nitrovasodilator nitroglycerin in two groups of rats (n=6, both groups). One group was pretreated with losartan, and the other was not.
DPMA

Fig 3. Bar graphs show effect of the A2-selective adenosine agonist DPMA without (Vehicle, open bars, n=6) and with (Losartan, filled bars, n=6) losartan pretreatment (10 mg/kg IV) on renal, mesenteric, and hindquarter vascular resistances. Data are mean±SEM. DPMA dose-dependently reduced vascular resistance in all three beds, and the response in the renal and mesenteric circulations but not the hindquarters was potentiated by losartan pretreatment. *Significant (P<.05) difference from vehicle by unpaired t test.

Results

Intravenous Dose-Response of DPMA With and Without Losartan Pretreatment

Baseline mean arterial pressure and heart rate were 108±4 mm Hg and 404±25 beats per minute, respectively, in the non-losartan-pretreated group. The baseline values before losartan treatment in the other group were not different (107±4 mm Hg and 381±17 beats per minute for mean arterial pressure and heart rate, respectively). Blockade of AT1 receptors with 10 mg/kg IV losartan decreased arterial pressure. Renal, mesenteric, and hindquarters vascular resistances decreased in response to dosing with losartan (Fig 1, Losartan I group).

Increasing doses of DPMA produced dose-related reductions in mean arterial pressure that were significant at 3, 10, and 30 µg/kg. As mean arterial pressure decreased, there were corresponding increases in heart rate, which were significant at 10 and 30 µg/kg (Fig 2, open bars).

The DPMA-induced depressor responses were the result of decreases in vascular resistance in the three vascular beds measured. Significant reductions in renal and hindquarters vascular resistances were observed at 3, 10, and 30 µg/kg IV DPMA, whereas significant reductions in mesenteric vascular resistance occurred at 10 and 30 µg/kg IV DPMA (Fig 3, open bars).

Administration of the adenosine receptor antagonist CGS 15943 completely reversed the effects of DPMA. Mean arterial pressure returned to 5±2 mm Hg above baseline, and heart rate returned to 7±5 beats per
FIG 4. Bar graphs show effect of the calcium channel blocker nifedipine without (Vehicle, open bars, n=6) and with (Losartan, filled bars, n=6) losartan pretreatment (10 mg/kg IV) on mean arterial pressure (MAP) and heart rate (HR). Data are mean±SEM. Nifedipine dose-dependently reduced MAP. Pretreatment with losartan tended to attenuate the depressor response, although the effect was not statistically significant. *Significant (P<.05) difference from vehicle by unpaired t-test.

Intravenous Dose-Response of Nifedipine With and Without Losartan Pretreatment

Baseline mean arterial pressure and heart rate in the losartan-treated and nonpretreated groups that received nifedipine were not different (114±3 versus 107±4 mm Hg, respectively, and 337±14 versus 334±9 beats per minute, respectively). Mean arterial pressure decreased after dosing with 10 mg/kg IV losartan. The vascular resistances in the three beds decreased in response to dosing with losartan (Fig 1, Losartan II group).

Increasing intravenous doses of nifedipine from 3 to 100 µg/kg resulted in a dose-related reduction in mean arterial pressure (Fig 4) and vascular resistance (Fig 5). The magnitude of these reductions was comparable to that obtained with DPMA. Whereas pretreatment with losartan potentiated the effects of DPMA, the responses to nifedipine either were not affected or were attenuated by losartan pretreatment. The depressor responses to nifedipine tended to be attenuated by losartan pretreatment, although the attenuation was not statistically significant. The reduction in renal vascular resistance in response to 100 µg/kg IV nifedipine was significantly less in the losartan-treated group compared with nonpretreated animals (−18±2% versus −25±2% minute below baseline, neither significantly different from control. Likewise, renal and hindquarters vascular resistances returned to levels not different from control after CGS 15943 administration (+9±4% and 0±4% of control, respectively). Mesenteric vascular resistance after CGS 15943 was +13±4% of control, a significant rebound increase above control.

Pretreatment with 10 mg/kg IV losartan significantly potentiated the depressor and renal and mesenteric vascular resistance responses to DPMA (Figs 2 and 3, closed bars versus open bars). The depressor responses to DPMA were potentiated at 1, 3, and 10 µg/kg, whereas the depressor response at 30 µg/kg was not different between losartan-treated and nonpretreated animals (Fig 2). The DPMA-induced reductions in renal vascular resistance were significantly greater in losartan-treated animals at 1 and 3 µg/kg but not at 10 or 30 µg/kg, whereas the reductions in mesenteric vascular resistance were potentiated by losartan treatment at 3 and 10 µg/kg. Interestingly, there were no significant differences in the hindquarters vascular resistance responses between the losartan-treated group and the nonpretreated group at any DPMA dose (Fig 3).
of control, respectively; \( P < .05 \). Likewise, nifedipine-induced reductions in mesenteric vascular resistance were significantly less in the losartan-treated group compared with the nonpretreated group at 3, 30, and 100 \( \mu g/kg \) IV nifedipine (100 \( \mu g/kg \); losartan-treated versus nonpretreated, \(-29\pm4\% \) versus \(-39\pm2\% \) of control; \( P < .05 \)). As with DPMA, losartan pretreatment did not affect the reductions in hindquarters vascular resistance in response to nifedipine.

**Intravenous Dose-Response of Nitroglycerin With and Without Losartan Pretreatment**

Baseline mean arterial pressure and heart rate in the nitroglycerin-dosed groups were also not different (109\pm4 mm Hg and 366\pm16 beats per minute in the non-losartan-treated group versus 115\pm4 mm Hg and 362\pm13 beats per minute in the losartan-treated group). Administration of losartan decreased mean arterial pressure, renal, mesenteric, and hindquarters vascular resistances decreased after administration of losartan (Fig 1, Losartan III group).

Increasing doses of nitroglycerin from 0.3 to 10 \( \mu g/kg \) IV reduced mean arterial pressure (Fig 6) and regional vascular resistances (Fig 7). These reductions were comparable to those obtained with DPMA. However, unlike DPMA and nifedipine, pretreatment with losartan did not alter any of the responses to increasing doses of nitroglycerin. Neither the depressor (Fig 6) nor the vasodilator (Fig 7) responses to nitroglycerin were affected.

**Intravenous Dose-Response of DPMA With and Without Nitroglycerin Pretreatment**

Baseline mean arterial pressure and heart rate in the two groups were not different (107\pm6 mm Hg and 299\pm15 beats per minute in the non-nitroglycerin-infused rats versus 115\pm6 mm Hg and 314\pm14 beats per minute in the nitroglycerin-infused group). The decrease in mean arterial pressure with nitroglycerin infusion was not different from that observed after losartan treatment. Renal, mesenteric, and hindquarters vascular resistances decreased with nitroglycerin infusion (Fig 1, Nitroglycerin group).

As shown in Fig 8, nitroglycerin infusion tended to attenuate the depressor responses to DPMA, which were significant at the 10 \( \mu g/kg \) dose of DPMA. Likewise, nitroglycerin infusion also appeared to blunt the vasodilator responses to DPMA (Fig 9). However, this effect was only significant in the renal vasculature at the 10 \( \mu g/kg \) dose of DPMA. As opposed to the significant potentiation of DPMA-mediated responses by losartan, nitroglycerin infusion had little, if any, effect. The only significant interaction observed was a slight attenuation of the DPMA-mediated depressor and renal vasodilator effects.

**Discussion**

Blockade of AT\(_1\) receptors in these experiments resulted in significant reductions in mean arterial pressure. Vascular resistances in the renal, mesenteric, and hindquarters circulations were also reduced. Note that there appears to be a regional difference in the vasodilator response to losartan, in that the rank order of sensitivity was renal to mesenteric to hindquarters. Therefore, these data indicate that a substantial level of angiotensin-mediated vascular tone is present in the model used in these studies, most likely a result of the anesthetic and surgical manipulations. The differences in the regional vasodilation indicate that angiotensin-mediated vascular tone is highest in the renal vasculature, which is greater than that in the mesenteric vasculature, which is, in turn, greater than that in the hindquarters. In this regard, the experiments with DPMA, nifedipine, and nitroglycerin represent a comparison of vascular reactivity to these agents between animals with substantial angiotensin-mediated tone and those with no AT\(_1\)-mediated tone. Removal of AT\(_1\)-mediated vascular tone by losartan treatment potentiated DPMA-induced reductions in mean arterial pressure and vascular resistance in the
renal and mesenteric vasculatures. The lack of effect in the hindquarters may be due to the lower sensitivity of this vascular bed to AT1 blockade compared with the other beds. Losartan pretreatment did not alter the depressor or vasodilator responses to nitroglycerin, and the responses to nifedipine were either unaffected or attenuated, indicating a selective interaction between Ang II and the dilator agent based on the mechanism of action of the specific agent. Also, the effect of losartan on DPMA was not due to alteration in baseline arterial pressure, because the responses to DPMA were not potentiated when arterial pressure was similarly lowered with nitroglycerin.

The adenosine agonist used in these studies, DPMA, is selective for the A2 subtype of adenosine receptors. Previous studies have shown this compound to be more than 13-fold selective for A2 over A1 receptors.14 Stimulation of A2 receptors has been shown to increase renal renin release,15 and administration of A2-selective agonists results in an increase in plasma renin activity.16 A recent report has demonstrated that infusion of adenosine into the forearm resulted in an increase in the local production of angiotensin.7 Both β-adrenergic receptor activation and A2 receptor stimulation are generally understood to exert their effects through increases in the second messenger cAMP. These data, taken together, suggest that the stimulation of circulating and local vascular Ang II production by adenosine is mediated via activation of the A2 adenosine receptor subtype.

Blockade of AT1 receptors potentiated the depressor and vasodilator actions of DPMA, suggesting that angiotensin blunts the activity of A2 adenosine receptor stimulation. Previous reports have demonstrated that angiotensin can attenuate the increases in intracellular cAMP stimulated by a variety of agents.21-25 Classically, the vasorelaxation mediated by A2 receptor activation is a result of a receptor/G protein–coupled reaction, which increases intracellular cAMP.10-13 It is conceivable that Ang II attenuates A2-mediated vasodilation through an inhibition of the A2-mediated increases in intracellular cAMP. The mechanism by which losartan pretreatment leads to increased potency of DPMA may be that blockade at AT1 receptors blocks Ang II–mediated inhibition of increased cAMP stimulated by DPMA, allowing full expression of the vasorelaxant potency of the compound.

**FIG 6.** Bar graphs show effect of the nitrovasodilator nitroglycerin without (Vehicle, open bars, n=6) and with (Losartan, filled bars, n=6) losartan pretreatment (10 mg/kg IV) on mean arterial pressure (MAP) and heart rate (HR). Data are mean±SEM. Nitroglycerin dose-dependently reduced MAP. Pretreatment with losartan had no effect.
Fig 7. Bar graphs show effect of the nitrovasodilator nitroglycerin without (Vehicle, open bars, n=6) and with (Losartan, filled bars, n=6) losartan pretreatment (10 mg/kg IV) on renal, mesenteric, and hindquarter vascular resistances. Data are mean±SEM. Nitroglycerin dose-dependently reduced vascular resistance in all three vascular beds. Losartan pretreatment had no effect on nitroglycerin-induced reductions in vascular resistance.

A recent report demonstrated that the depressor response to K⁺ channel activators, including aprakalim, cromakalim, and diazoxide, was potentiated by losartan pretreatment.26 These experiments consisted of 20-minute infusions of the potassium channel activators. At the end of the infusion, plasma renin activity was shown to be increased threefold over baseline levels. Therefore, the authors concluded that the enhanced depressor activity of the potassium channel activators after losartan pretreatment was due to the removal of an elevated constrictor activity of Ang II, allowing full expression of the depressor response. Although adenosine receptors have been shown to be positively linked to K⁺ channels,14 the receptor subtype involved is A₁ and not A₂.27 Therefore, it is unlikely that the mechanism of the potentiation of DPMA by losartan involves K⁺ channel activation. Thus, losartan pretreatment may also potentiate the depressor responses to other types of dilator agents not studied in these experiments.

Pretreatment with losartan attenuated the vasodilator effects of the Ca²⁺ channel blocker nifedipine. Previous reports have shown that the pressor response to Ang II was diminished after administration of calcium channel blockers.28-31 Thus, one mechanism by which angiotensin elicits an increase in vascular smooth muscle tone is through activation of the L-type slow Ca²⁺ channels, thereby increasing Ca²⁺ flux into the cytoplasm. Assuming that some level of basal calcium flux is mediated by endogenous levels of angiotensin, blockade of AT₁ receptors should result in a decreased calcium flux. Thus, a lower level of calcium flux, as caused by losartan treatment, would decrease the efficacy of blocking the channel. Hence, the vasorelaxant activity of nifedipine should be attenuated when AT₁ receptors are previously blocked.

Another potential explanation for the altered responses to DPMA and nifedipine with losartan pre-
observed with nitroglycerin. Therefore, the decreased depressor effect or reduction in vascular resistances with nitroglycerin argue against this possibility. Pretreatment with losartan had no effect on the response to DPMA at 10 ng/kg. Significant (P<.05) differences in the decrease of renal, mesenteric, and hindquarter vascular resistance were already somewhat dilated, the response to losartan was not different before dosing with DPMA or nifedipine. Because the mechanism of action of nitroglycerin is through activation of guanylate cyclase and the subsequent increase in intracellular cyclic GMP and no known effect of angiotensin is mediated by this second messenger, the lack of an interaction between losartan and nitroglycerin would be predicted.

In summary, blockade of AT1 receptors increased the vasodilator and depressor potency of DPMA but not of nifedipine or nitroglycerin. Stimulation of A1 adenosine receptors increases Ang II levels, and these increased levels presumably blunt the vasodilator potency of A2 adenosine agonists. It is not clear from the present experiments whether the source of Ang II is from the circulation or of local vascular origin. The recent demonstration of adenosine stimulating the production of Ang II in human forearm clearly indicates that the origin can be local vascular tissue. Regardless of the source of Ang II, these studies demonstrate that Ang II interacts selectively with vasodilator-mediated vasodilation and that this interaction appears to be dependent on their mechanism of action.

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