Analogs of Phosphatidylcholine: α-Adrenergic Antagonists from the Renal Medulla
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SUMMARY Antihypertensive polar renomedullary lipid (APRL), a conglomerate of l-0-alkyl-2-acetoyl-glicero-3-phosphocholine analogs, was tested in 4- to 6-week-old spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats using microcirculatory techniques. APRL (0.5 μg/ml), when added to the solution bathing the cremaster muscle, caused significant changes in the diameter, red blood cell velocity, and blood flow in both groups of rats, for arterioles and venules. Arteriolar changes in diameter were significantly greater (p < 0.05) in SHR than in WKY. Micropipette application of APRL indicated a dose-dependent response for arterioles and venules in both groups. Moreover, the potent nature of this compound was demonstrated. Relative potency of APRL given intravenously was tested in 10- to 12-week-old SHR and WKY. The response curve was shifted significantly to the left for SHR (p < 0.01). APRL interaction with known controllers of blood flow was tested in SHR. Blockade of cholinergic, β-adrenergic, or histaminergic receptors did not inhibit APRL action. Blockade of prostaglandin or bradykinin synthesis did not prevent depression of blood pressure by APRL. APRL (40 μg/kg) inhibited (p < 0.001) the pressor response to norepinephrine (1–10 μg/kg) but not to angiotensin II (4 μg/kg). The present study provides direct evidence that APRL is a vasodilator with increased potency in SHR hypertension. The acute vascular response may be mediated by alpha-adrenergic antagonism. (Hypertension 3: 460-470, 1981)

KEY WORDS • l-aklyl-2-acetoyt-glycero-choline • alpha-adrenergic antagonist • spontaneously hypertensive rat • norepinephrine antagonist • cremaster muscle • microcirculation

SEVERAL lines of evidence support the hypothesis that the renal medulla plays an endocrine-like role in the prevention of hypertension.1-3 Two lipid compounds displaying depressor activity have been extracted from the renal medulla, one neutral and the other polar. The antihypertensive polar renomedullary lipid (APRL) has been shown to elicit both an acute and a prolonged reduction in blood pressure (BP). We designed the present experiments to clarify the acute vascular response to APRL. The phenomena observed could provide a means of evaluating the mechanism of the prolonged action of these compounds.

Recently, APRL was demonstrated to consist of a conglomerate of l-0-alkyl ether analogs of phosphatidylcholine having an acetoyl at the second position.* The ether chains consisted of C16:0 (67%), C16:1 (16%), C18:1 (11%), C18:0 (4%), and C15:0 (2%).

We conducted microcirculatory experiments to provide direct evidence that APRL is a vasodilator. In addition, we evaluated the relative potency and interaction with known controllers of blood flow for which suitable blocking agents and agonists are available.

Materials and Methods

Microcirculatory Experiments

Microcirculatory techniques were used to observe the vascular response of normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rat (SHR)7 microvessels to lapine APRL extracted by procedures previously described.8 At the time these experiments were performed, the APRL preparation contained only 10% of the active alky ether conglomerate. The
remainder of the preparation consisted of inactive material, mostly 1-O-acetyl-2-acetoyl-glycero-3-phosphocholine. Thus, the factor 0.1 should be applied to the dose schedules of APRL used in the present experiments. The doses used in the present experiments were derived by weighing the APRL preparation in a Cahn Gram Balance (Cahn Instrument Company, Paramount, California).

The cremaster muscle preparation as described by Baez was on 4- to 6-week-old WKY and SHR. A total of 39 WKY ranging in weight from 57 to 123 g (average, 91 g) and 38 SHR weighing from 58 to 145 g (average, 93 g) were used to record the responses of cremasteric microvessels to APRL administered by three different methods.

Prior to surgery and experimentation, each animal was anesthetized with a solution of 2% chloralose and 10% urethane in saline, given intraperitoneally (i.p.) at a dose of 6 ml/kg. The animal was then placed on a heating pad to maintain body temperature while surgery was performed. The cremaster muscle, once exposed, was kept moist at all times by dripping warm Normosol directly onto the preparation. Five ties were then placed on the circumference of the cremaster muscle so that it could be evenly spread over a lucite pedestal.

For measuring BP during experimentation, the right carotid artery was cannulated, using PE 10 tubing. The right external jugular vein was cannulated with PE 10 tubing for i.v. injections when APRL was administered by this method.

Following surgery, the animal was placed on a specially designed platform that could be mounted on a microscope stage. The cremaster was lightly stretched over a lucite pedestal on this platform so that the microvessels of the muscle preparation could be observed. Krebs-Henseleit solution equilibrated with 5% CO₂ and 95% nitrogen gas was continuously suffused over the muscle. The temperature of the solution bathing the cremaster was maintained at 34°C, and the pH was found to be 7.4 when analyzed on several occasions. The suffusate was drawn from the bath by means of a vacuum pump to maintain the proper fluid level over the preparation. The animal's body temperature was maintained at approximately 37°C by a heating element on the platform beneath the animal, which was controlled by a feedback circuit. The carotid artery cannula was connected to a BP transducer, and the animal's BP was recorded on a Brush chart recorder. If necessary, additional anesthetic was administered during the course of the experiment, in increments of 0.1 ml.

Following a 30-minute stabilization period, the cremaster muscle preparation was transilluminated and visualized through binocular eyepieces on the microscope or on a video monitor. The video monitor was calibrated (× 1660) so that accurate measurements of the internal diameter of the blood vessels could be made using calipers. Part of the light passing through the muscle preparation was deflected to a dual-slit apparatus to measure red blood cell (RBC) velocity by the two-slit photometric method of Wayland and Johnson. Blood flow was calculated using the vessel diameter, RBC velocity, and the correction factor of Baker and Wayland.

Only blood vessels that could be clearly visualized for measuring internal diameters and that had fairly stable RBC velocity profiles were used for observation. Vessels on the medial aspect of the cremaster preparation were recorded more frequently since this side of the muscle is thinner and more easily transilluminated and visualized. Generally, a number of vessels could be recorded from each preparation. Vessels were classified as arterioles or venules according to flow direction. Deterioration of the preparation could be determined by the appearance of red blood cells exterior to the lumen of the cremasteric microvessels, by accumulation of leukocytes along the internal walls of these vessels, and by fasciculation of the muscle fibers. Once the preparation showed sufficient signs of deterioration, the animal was sacrificed.

In the first experiment, APRL (10 μg/ml saline) was added through a side arm in the superfusion system by an infusion pump. The concentration of APRL reaching the cremaster muscle was approximately 0.5 μg/ml for 30 seconds. Vessel diameter and velocity changes were recorded continuously for 1 minute prior to the addition of APRL and for 4 minutes following the initial exposure.

In the second experiment, APRL was administered using glass micropipettes pulled and ground so that the tips were from 2 to 4 μm in outside diameter. The pipettes were filled with APRL (0.025, 0.25, 2.5, and 25.0 μg/ml saline) or saline and placed in a pipette holder. This was connected to a 50 μl syringe held in a Hamilton microliter dispenser. Depression of the trigger mechanism on this dispenser delivered a volume of 1 μl from the tip of the pipette. With a micromanipulator, the pipette was lowered onto the cremaster preparation, and its tip placed as close as possible to the vessel being measured, without occluding its lumen. Diameter changes were recorded for a 1-minute period prior to application of APRL, and for 4 minutes following application.

In the third experiment, APRL was administered through a cannula in the external jugular vein in doses of 0.01, 0.1, 1.0, 10, 50 and 100 μg/kg. Changes in vessel diameter and RBC velocity were measured for a time course similar to that in the superfusion and microinjection experiments.

In the event that administration of APRL did not cause a vessel to dilate, a few drops of 1 mM adenosine solution were added to the muscle bath to determine whether the vessel in question was already maximally dilated. If a vessel did not dilate in response to adenosine, it was assumed to be maximally dilated. If a vessel responded to adenosine, the data were included in the results.

The data were analyzed at 10-second intervals during the 1-minute control period immediately prior to administration of APRL, and for 4 minutes after application. The data for each vessel's response were compared to the averaged control data to calculate the percentage change in each parameter during the
response. The normalized data were then averaged within groups for each 10-second time interval and represented as a mean value plus or minus the standard error (± SE).

An analysis of variance for multiple observations was run to determine if the average response for each group was different from the control, indicated by a significant interaction over time (p < 0.05), and to determine if WKY vessels responded differently from SHR vessels, indicated by a significant interaction between groups (p < 0.05). When analysis of variance indicated a significant interaction between groups, the Student's t test was used to determine which points were significantly different at the p < 0.05 level.

Dose-Response and Pharmacologic Blockade

In the series of experiments to determine if APRL interacts with any of the known controllers of blood flow, 10- to 12-week-old rats were used. They were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The femoral artery and external jugular vein were cannulated for BP measurements and i.v. injections respectively. Both cannulas were externalized through an incision in the skin at the back of the neck.

Following recovery from surgery, the dose-response relationship for APRL was determined in six conscious WKY and SHR. Doses of 0.01, 0.1, 1.0, 10, 50, and 100 μg/kg of APRL were given in 0.1 ml saline, followed by 0.1 ml saline to ensure that the APRL was flushed completely through the i.v. cannula. The BP was recorded for 10 minutes before the injection of APRL, to obtain a stable baseline with which to compare the response, and for 10 minutes following APRL administration.

Only SHR were used for the pharmacologic-blocking studies. The agonist for the dilating mechanism was administered to test its efficacy, then the appropriate blocking agent was given, followed by the agonist a second time to test the pharmacologic blockade. Finally, APRL was administered to determine if its hypotensive action was abolished or diminished.

All drugs were administered in a volume of 0.1 ml saline followed by 0.1 ml saline. The following agents were administered to six SHR on separate days to determine if they blocked the hypotensive action of APRL. Atropine (1 mg/kg) was used to block cholinergic receptors; blockade was tested using acetylcholine (10 μg/kg). Propranolol (0.1 mg/kg) was used to determine if the polar lipid (APRL) causes vasodilation by interacting with β-adrenergic receptors; blockade was tested using isoproterenol (1 μg/kg). To see if APRL acts through a histaminergic mechanism, chlorpheniramine (4 mg/kg), an H1 receptor blocker, was combined with cimetidine (12 mg/kg), an H2 receptor blocker. Histamine (1 μg/kg) was given to test blockade of vascular histamine receptors. Indomethacin (4 mg/kg) was used to rule out the possibility of APRL causing dilation by the synthesis and release of prostaglandins. Aprotinin (10,000 U/kg), the kallikrein inhibitor, was given to see if the polar lipid stimulated bradykinin synthesis. After each blockade, APRL was given in a dose found to elicit a maximal drop in BP in the SHR (10 μg/kg).

The average BP drop elicited by APRL following each pharmacologic blockade was determined and compared to the response seen when APRL was given alone, using the paired t test.

To determine if APRL lowers the BP by blocking endogenous constrictor agents, the following protocol was used. First, the pressor agent was given to test its efficacy (norepinephrine, 1 μg/kg, or angiotensin II, 4 μg/kg). APRL was then administered in a relatively large dose (40 μg/kg), which was capable of depressing the BP for several minutes. The constrictor agent was given again while the BP was lowered to see if APRL blocked its pressor effect. The responses to the constrictor agent before and after APRL administration were compared using the paired t test.

All dosages for pharmacologic blocking agents and agonists used in these studies have been shown to be effective in laboratory animals or man.11,12

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Percent change in diameter (± SE) for arterioles and venules in response to addition of APRL to the suffusing solution for SHR (solid line) and WKY (dashed line). The cremaster muscle preparation was exposed to APRL (0.5 μg/ml suffusate) from 0 to 30 seconds, designated by the horizontal bar below the abscissa. This caused arterioles and venules of both groups to dilate significantly. The SHR arteriolar diameter changes were significantly greater (p < 0.05) than WKY responses from 50 to 70 seconds. There was no difference between groups for diameter changes in venules. Control data for superfusion experiments are given in table 1.
Results

Microcirculatory Experiments

Superfusion of APRL

The results obtained from monitoring the responses of SHR and WKY arterioles and venules to the addition of APRL to the suffusing solution are shown in figures 1–3. All results are expressed as the percent change from control values, which were determined by averaging six points taken at 10-second intervals during the minute preceding the addition of APRL. The average control diameter, red blood cell velocity, flow, mean arterial pressure (MAP), number of vessels observed, and number of animals for SHR and WKY arterioles and venules are shown in table 1. The MAP was significantly greater ($p < 0.01$) in SHR when compared to WKY. No differences were indicated between SHR and WKY for other hemodynamic parameters during the control period.

Following exposure to APRL from time zero to 30 seconds, designated by the horizontal bar below the abscissa, both SHR and WKY cremasteric arterioles dilated rapidly (fig. 1). The WKY arterioles reached a maximum increase in diameter of $6.0\% \pm 1.7\%$ at 40 seconds. The SHR arterioles attained a maximum dilation of $20.3\% \pm 3.8\%$ at 70 seconds. WKY responses returned to control values within 3 minutes after APRL exposure. SHR responses had a much longer time course, taking approximately 8 minutes to return to control levels. Analysis of variance for multiple observations showed a significant interaction over

![Figure 2. Percent change in RBC velocity (± SE) for arterioles and venules in response to superfusion of APRL for SHR (solid line) and WKY (dashed line). Velocity changes for arterioles and venules of both groups were significantly different from control data (table 1). No significant difference between groups was indicated by analysis of variance for arterioles or venules.](image1)

![Figure 3. Percent change in flow (± SE) for arterioles and venules in response to superfusion of APRL for SHR (solid line) and WKY (dashed line). Flow changes were significantly different from control data (table 1) for arterioles and venules of both groups. No significant difference between groups was indicated by analysis of variance for arterioles or venules.](image2)
time for both groups, as well as significant interaction between groups during this response. The Student's $t$ test indicated that SHR arteriolar diameter changes were significantly greater ($p < 0.05$) than those of WKY vessels from 50 to 70 seconds. The responses were not analyzed beyond 4 minutes after onset of APRL exposure. There was no difference in the diameter changes between SHR and WKY for venules. Analysis of variance showed a significant interaction over time for both groups, thus indicating that the response was different from the control period for WKY and SHR venules. The WKY venules reached a maximum of 8.2% ± 1.0% at 100 seconds. The diameters for both groups of venules returned to control values within 4 minutes after exposure to APRL, as shown in figure 1.

Considerable variability was observed for RBC velocity changes in arterioles and venules when the cremaster muscle preparations were exposed to APRL in the suffusing solution. Analysis of variance showed no difference between SHR and WKY, although there were significant velocity changes over time for the arterioles and venules in both groups. SHR arterioles showed an immediate pronounced fall in velocity upon exposure to APRL, reaching $-14.0\% \pm 4.0\%$ at 30 seconds. This was followed by a somewhat slower return to control levels. WKY arterioles first showed an increase in velocity to $10.8\% \pm 3.7\%$ at 10 seconds, followed by a rapid fall to $-4.4\% \pm 4.1\%$ at 40 seconds.

The RBC velocity changes for venules were remarkably similar between SHR and WKY. Venules from both groups of experimental animals responded with a slight increase in velocity during APRL exposure. SHR RBC velocity in venules reached a maximum increase of $9.3\% \pm 5.3\%$ at 30 seconds, while WKY responses increased to $7.1\% \pm 7.4\%$ above control values at 30 seconds. The RBC velocity changes are illustrated in figure 2.

While it appears that SHR arteriolar blood flow increased more than that of WKY arterioles, this difference is not statistically significant, as indicated by analysis of variance. SHR arterioles reached a maximum increase in blood flow of $51.1\% \pm 14.7\%$ at 70 seconds. WKY arterioles increased flow to a maximum of $16.6\% \pm 12.4\%$ at 100 seconds. Blood flow in both groups of arterioles returned to control levels within 4 minutes (fig. 3).

Flow changes in venules for WKY and SHR were nearly identical. SHR venules attained a maximum increase in flow of $21.3\% \pm 11.3\%$ at 70 seconds, while WKY venules increased flow to a maximum of $20.5\% \pm 14.6\%$ at 30 seconds. Flow returned to control levels by 4 minutes in both groups of venules (fig. 3).

No change in MAP was observed in response to APRL superfusion over the cremaster muscle preparation in SHR or WKY.

**Microplpette Application of APRL**

The results from the experiments utilizing micropipette application of APRL are shown in figures 4–6. Table 2 gives the average control diameters, MAP, number of vessels observed, and the number of animals used. Arterial pressures were significantly greater ($p < 0.01$) in SHR when compared to WKY.

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**TABLE 1. Control Data for Superfusion of APRL**

<table>
<thead>
<tr>
<th></th>
<th>Arterioles</th>
<th>Venules</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Diameter (μm)</td>
<td>28.7 ± 3.2</td>
<td>28.5 ± 2.5</td>
</tr>
<tr>
<td>RBC velocity (mm/sec)</td>
<td>6.3 ± 1.4</td>
<td>4.6 ± 1.0</td>
</tr>
<tr>
<td>Flow (nl/sec)</td>
<td>3.0 ± 1.2</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>MAP (mm/Hg)</td>
<td>115 ± 4*</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>No. of vessels</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>No. of rats</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

*Significantly greater value ($p < 0.01$)

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**TABLE 2. Control Data for Micropipette Application of APRL**

<table>
<thead>
<tr>
<th></th>
<th>Arterioles</th>
<th>Venules</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Diameter (μm)</td>
<td>37.0 ± 2.4</td>
<td>30.0 ± 2.1</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>118 ± 3*</td>
<td>92 ± 2.1</td>
</tr>
<tr>
<td>No. of vessels</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>No. of animals</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

*Significantly greater value ($p < 0.01$)
FIGURE 4. Percent change in diameter for SHR (solid line) and WKY (dashed line) arterioles in response to APRL applied by micropipette at time zero in the doses shown. All doses were given in a volume of 1 μl saline onto the surface of the vessel being monitored. All doses caused significant diameter changes except 0.025 ng when applied to WKY arterioles. There were no other differences between groups indicated by analysis of variance. Control data are given in table 2.

FIGURE 5. Percent change in diameter for SHR (solid line) and WKY (dashed line) venules in response to APRL applied by micropipette at time zero in the doses shown. All doses caused significant changes in diameter from control data for both groups. There were no differences between groups indicated by analysis of variance.
The diameter changes seen when APRL was applied to SHR and WKY arterioles in doses of 0.025, 0.25, 2.5, and 25 ng at time zero are illustrated in figure 4. Analysis of variance indicated no significant interaction between the groups for any of these doses. All doses caused significant dilation ($p < 0.05$) in both groups of animals except 0.025 ng when applied to WKY arterioles.

The responses seen when APRL was applied to SHR and WKY venules in the same doses as those used for arterioles are shown in figure 5. No significant interaction was indicated between SHR and WKY by analysis of variance. Significant increases in diameter were seen in both groups for all doses.

By taking the maximum response from the averaged data in figures 4 and 5, the dose–response curves for arterioles and venules were compiled. As with superfusion, micropipette application of APRL produced no change in MAP (fig. 6).

**Intravenous Injection of APRL**

Attempts to monitor the diameter changes of microvessels in response to i.v. administration of APRL were unsuccessful. Small doses that did not lower MAP produced no visible diameter changes in SHR or WKY microvessels. Doses that caused even the slightest drop in BP stopped blood flow in the cremaster preparation. It was then impossible to determine if the diameter changes were due to APRL acting upon those vessels or if the changes observed were simply reflex responses to decreased blood flow. Therefore, no data are presented for these experiments. It should be noted that these results did not make the vascular bed inappropriate for experiments that were performed.

**Dose-Response and Pharmacologic Blockade**

**Intravenous Dose-Response Relationship**

The SHR used in these experiments had a MAP of $165 \pm 5$ mm Hg and an average weight of $269 \pm 3$ g. The WKY had a MAP of $122 \pm 2$ mm Hg, with an average weight of $233 \pm 4$ g. Blood pressure and body weight were significantly greater ($p < 0.01$) in SHR when compared to WKY. The results from these experiments are shown in figure 7. The response curve for SHR is shifted to the left. Three of six SHR responded to 0.1 $\mu$g/kg APRL, with an average fall in MAP of approximately 10 mm Hg. Including the animals that did not respond, the average drop in BP was $5 \pm 2$ mm Hg for this dose. The WKY showed no response to 0.1 $\mu$g/kg APRL. Four of six SHR responded to 1.0 $\mu$g/kg APRL, with an average fall in MAP of about 17 mm Hg. When the two animals that did not respond were included in the calculations, the average BP decrease was $11 \pm 4$ mm Hg. The WKY did not respond to this dose. All six SHR responded to 10 $\mu$g/kg APRL, with a mean maximum fall in MAP ($-57 \pm 4$ mm Hg) of short duration (2 minutes). None of the six WKY responded to this dose. When the APRL dose was increased to 50 $\mu$g/kg, all six SHR responded ($-62 \pm 4$ mm Hg), and the BP returned to control levels much more slowly (10 minutes). Two of six WKY responded to 50 $\mu$g/kg APRL with a fall in MAP of about 32 mm Hg. The average for all six WKY was $-11 \pm 7$ mm Hg. All six WKY responded to APRL in a dose of 100 $\mu$g/kg. The average fall in MAP was $58 \pm 2$ mm Hg, with a short duration (2 minutes). This dose was not used in SHR because of a limited supply of APRL and fear of overdosing these animals. Typical responses to i.v. APRL are shown in figure 8.

![Figure 6. Dose-response relationship for arterioles and venules for SHR (solid line) and WKY (dashed line) in response to micropipette application of APRL. These points were compiled from the maximum responses shown in figures 4 and 5. Arterioles and venules of both groups responded in a dose-dependent fashion. No significant interaction was indicated between groups for all doses, except that WKY arterioles did not respond to 0.025 ng.](http://hyper.ahajournals.org/content/3/4/466/F6.large.jpg)
ALKYL ETHER AS ADRENERGIC ANT AGONIST/Smith et al.  467

Since SHR were found to be more sensitive to the acute depressor action of APRL, they were used in the pharmacologic-blocking studies. A dose of 10 μg/kg APRL was used since it consistently produced a maximum BP fall in SHR.

Blockade of Endogenous Dilating Systems

Acetylcholine (10 μg/kg) given prior to blockade with atropine (1 mg/kg) caused an average decrease in MAP of 60 ± 1 mm Hg in six conscious SHR. Following blockade, the average depressor effect was −3 ± 2 mm Hg. APRL (10 μg/kg) given during blockade of cholinergic receptors caused an average drop in MAP of 55 ± 2 mm Hg (fig. 9A).

Isoproterenol (1 μg/kg), the β-adrenergic agonist, caused a fall of 46 ± 5 mm Hg in MAP. After blockade with propranolol (0.1 mg/kg), isoproterenol produced no response. APRL (10 μg/kg) given during blockade of β-adrenergic receptors depressed MAP 49 ± 5 mm Hg (fig. 9B).

Histamine (1 μg/kg) reduced MAP 28 ± 2 mm Hg. The H₂ receptor blocker, chlorpheniramine (4 mg/kg), when given with cimetidine (12 mg/kg), the H₂ receptor blocker, produced a slight increase in BP (+15 ± 2 mm Hg). No response was seen to histamine following blockade. APRL caused an average reduction of 58 ± 10 mm Hg in MAP (fig. 9C). Only one animal in six did not respond to APRL following blockade of histamine receptors.

Prostaglandin synthesis was inhibited using indomethacin (4 mg/kg). This did not block the acute depressor effect of APRL (−53 ± 4 mm Hg) (fig. 9D, left).

Bradykinin synthesis was inhibited using aprotinin (10,000 U/kg), the kallikrein inhibitor. The acute depressor effect of APRL (−56 ± 5 mm Hg) was not blocked by aprotinin (fig. 9D, right).

Interaction with Vasoconstrictor Systems

Norepinephrine (NE) in a dose of 1 μg/kg caused MAP to increase 42 ± 3 mm Hg, transiently. Angiotensin II (All), given in a dose of 4 μg/kg, caused BP to rise 60 ± 3 mm Hg, transiently. APRL (40 μg/kg) lowered MAP to a level between 90 and 100 mm Hg (−62 ± 4 mm Hg) for several minutes. While the BP was reduced, the vascular response to NE (1 to 10 μg/kg) was totally blocked. It did not return until MAP had returned to near normal levels. All still caused an increase in MAP (+61 ± 3 mm Hg). These results are illustrated in figure 10. Typical responses are shown in figure 11.

Discussion

The changes in diameter seen during the microcirculatory experiments provide direct evidence that APRL is a potent dilator of arterioles and venules in both normotensive and hypertensive rats. Furthermore, significant changes in RBC velocity were seen in both SHR and WKY when this parameter was monitored.
Although no significant differences were observed between groups in the control data for the superfusion experiment (table 1), a tendency for RBC velocity and blood flow to be increased in SHR arterioles and decreased in venules was apparent. This is in agreement with previous observations of decreased numbers of small arterioles and increased numbers of small venules in SHR.11

The results of the superfusion experiments indicate that APRL dilates SHR arterioles to a significantly greater extent than WKY arterioles when administered by this method. Interestingly, SHR arterioles dilated essentially the same amount as WKY arterioles when the technique of micropipette application was used. One possible explanation for this discrepancy is that adding APRL to the solution bathing the entire cremaster muscle exposed all vessels of the preparation to this compound. This would cause the larger vessels of the preparation to dilate as well, and thus permit transmission of higher intravascular pressures to the smaller vessels that were being monitored. This would cause the smaller vessels that were dilating in response to APRL to dilate to a greater extent in SHR when compared to WKY, than if these vessels were exposed individually to minute quantities of APRL, as in experiments utilizing micropipette application. It is known that SHR microvessels have proportionally higher intravascular pressures than corresponding vessels in WKY.14

Experiments utilizing micropipette application of APRL indicate that the magnitude of dilation seen un-

![Figure 9](http://hyper.ahajournals.org/)

**Figure 9.** Shown is the APRL (10 μg/kg) interaction with endogenous dilating systems. Each graph shows the change in mean arterial pressure (MAP) ± SE (direction indicated by arrow) in response to agonists and blocking agents given in the order presented within graphs. Blockade of cholinergic (A), β-adrenergic (B), or histaminergic (C) receptors did not inhibit APRL action. Blockade of prostaglandin or bradykinin synthesis did not prevent depression of MAP by APRL (D). Six conscious SHR (10 to 12 weeks old) were used in each blocking study. Doses used are given in text.

![Figure 10](http://hyper.ahajournals.org/)

**Figure 10.** APRL (40 μg/kg) interaction with endogenous vasoconstrictors (A = norepinephrine; B = angiotensin II). Both graphs show the change in mean arterial pressure (MAP) ± SE (direction indicated by arrow) in response to pressor agents or APRL. The asterisk indicates significant difference (p < 0.001). Six conscious SHR (10 to 12 weeks old) were used in both experiments. Doses used are given in text.
under these conditions is dose-dependent for arterioles and venules in both groups of rats. Furthermore, this method indicates the potent nature of APRL in producing vasodilation since less than one nanogram applied directly onto the surface of arterioles and venules caused significant increases in diameter.

Venous dilation, seen both in the superfusion experiments and with micropipette application, may explain the fall in cardiac output seen when APRL is administered i.v. in large doses.4 The cessation of blood flow observed when microvascular hemodynamic parameters were monitored in response to i.v. APRL is consistent with the effect of this compound on venous capacitance and cardiac output. Smaller doses of APRL that cause significant reductions in BP cause only a slight decrease in cardiac output. This may be due to the effect of this compound on the resistance vessels, i.e., the small arteries and arterioles.

The results seen when BP changes were monitored in response to i.v. APRL indicate that this compound has increased potency in the SHR when compared to WKY. The mechanism responsible for the increased potency in SHR is not known.

Studies utilizing pharmacologic-blocking agents indicate that APRL does not cause dilation by interaction with cholinergic, β-adrenergic, or histaminergic systems. Furthermore, APRL does not produce vasodilation by the release of prostaglandins or bradykinin.

APRL appears to be an α-adrenergic antagonist since it blocks the pressor response to i.v. norepinephrine. It does not block the vascular effect of angiotensin II. Alpha-adrenergic blocking agents are known to be potent depressor agents; they are also known to cause venous dilation.15 The results presented in this report are consistent with APRL antagonizing the vascular effects of α1 or postsynaptic adrenergic stimulation.16

It has been known for some time that the vasculature demonstrates increased sensitivity to catecholamines in hypertension.17–21 Whether the kidneys play an inhibitory role in regulating vascular reactivity to these pressor agents is not yet known. This possibility should be entertained since APRL, although semisynthetic, is extracted from the renal medulla, is an α-adrenergic antagonist, has increased depressor potency in SHR hypertension, and apparently has been derived from the renal venous effluent of the isolated kidney.22

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