Regional Hemodynamic Effects of Antihypertensive Renomedullary Lipids in Conscious Rats

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SUMMARY Renomedullary tissue has been proposed to exert an antihypertensive endocrine-like action. The antihypertensive polar renomedullary lipids (APRL) and neutral renomedullary lipids (ANRL) are potential mediators of this action. We evaluated the blood pressure and regional hemodynamic responses to APRL administered peripherally (i.v.) and to the central nervous system (CNS) in normal rats and rats with sinoaortic deafferentation (SAD) to remove baroreflex buffering. Rats were chronically instrumented with Doppler flow probes for measurement of mesenteric, renal, and hindquarter vascular resistance, with arterial pressure and intravenous catheters, and with lateral cerebroventricular cannula for intracerebroventricular (i.c.v.) administration. Intravenous APRL (0.01 to 1.0 μg) produced a dose-dependent decrease in blood pressure, tachycardia, and dilation of all vascular beds studied. The dose-response relationships were shifted to the left in SAD animals. APRL administered i.c.v. had no effect on intact or SAD rats. Pressor and regional vasoconstrictor responses to norepinephrine, angiotensin, and vasopressin were markedly reduced in SAD animals during constant infusion of APRL. In a second group of conscious SAD animals instrumented for blood pressure and heart rate measurements, intravenous ANRL (500 μg) decreased both arterial pressure (-45 ± 16 mm Hg) and heart rate (-50 ± 16 bpm). When given i.c.v., however, ANRL (10–100 μg) had no significant effect on resting blood pressure or heart rate. These studies suggest that APRL and ANRL produce no significant cardiovascular effects that are mediated through the CNS. However, both lipids are potent depressor agents, and APRL exhibits a strong peripheral vasodilator action and nonspecifically reduces reactivity to vasoconstrictor agents.

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KEY WORDS • blood pressure regulation • vascular resistance • angiotensin II • vasopressin • norepinephrine • central nervous system action • sinoaortic deafferentation

RECENT evidence has led to the hypothesis that the kidney may exert an antihypertensive influence through a distinct endocrine-like system.

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ized is a highly polar glycerophospholipid, designated as antihypertensive polar renomedullary lipid (APRL). The derivation of biologically active APRL involves Vitride [NaAlH$_4$ (OC$_2$H$_5$ OCH$_3$)$_3$] reduction of the tissue extract and is thus termed semisynthetic. The other main lipid, designated antihypertensive neutral renomedullary lipid (ANRL), does not require Vitride reduction to unmask its biological activity in the extract ed state. Both APRL and ANRL lower arterial pressure when administered intravenously. In contrast to APRL, intravenous ANRL has been found to reduce heart rate in conscious rats in association with reduced blood pressure and increased efferent renal nerve activity. This absence of reflex tachycardia and increased renal nerve activity during ANRL-induced hypotension suggests that the lipids may act, in part, on the central nervous system (CNS) to produce their antihypertensive actions, although this has not been investigated.

To further characterize the mechanism by which APRL and ANRL reduce arterial pressure, we have developed techniques to measure regional blood flow in conscious, chronically instrumented rats. The studies were designed to 1) examine the overall hemodynamic effects of peripherally administered APRL; 2) determine whether the cardiovascular actions of APRL and ANRL depend, in part, on an action on the CNS; and 3) examine the influence of APRL on vascular reactivity.

Methods

Chronic Instrumentation

We used male Sprague-Dawley rats (275–350 g), which were housed in 25 x 17 x 8 cm opaque plastic cages. After pretreatment with atropine (1.3 mg/kg, i.p.), the rats were anesthetized with 0.1 ml/100 g i.m. of ketamine (100 mg/ml) to which 10 mg of acepromazine had been added. Supplemental anesthesia was given as required. Using aseptic procedures, we performed a midline laparotomy and carefully isolated 3 to 4 mm lengths of the superior mesenteric artery, lower abdominal aorta below the left renal artery, and the right or left renal artery under a dissecting microscope to avoid damage to nearby nerves. Miniaturized pulsed-Doppler flow probes were sutured in place around each vessel. A complete description of the construction and implantation of these flow probes has been reported elsewhere. The wire leads were brought beneath the skin to exit at the back of the neck, where they were soldered to a connector plug fixed to the animal's skull with jeweler's screws and dental cement. Before the connector plug was fixed, some animals received a 23-gauge stainless steel (s/s) lateral ventricular cannula which was stereotaxically positioned according to the following coordinates (in mm) relative to Bregma: caudal 0.8; lateral 1.1 to 1.2; ventral 6.0 from top of skull. A catheter (PE-10) was placed in the lower abdominal aorta via the left femoral artery for measurement of arterial pressure, and one or two catheters (PE-10) were placed in the lower abdominal vena cava via the right femoral vein for infusion of drugs. Catheters were exteriorized at the back of the neck. Animals were treated with 80,000 units penicillin, i.m. (Floccin, Bristol) and were allowed at least 3 days to recover from the surgery.

On the day of the experiment each rat was connected to a lightweight flexible spring that contained the flow probe wire connectors and arterial and venous connector lines. The spring-guarded connector line was suspended from the top of the animal’s home cage to allow freedom of movement during the experiment. Regional blood flow was measured with a pulsed-Doppler flowmeter (University of Iowa Bioengineering Facility). Mean arterial pressure (MAP) was electronically derived from a Century CP-01 pressure transducer, and heart rate (HR) was obtained with a Beckman 9857 B tachometer that was triggered from the arterial pressure pulse. Regional flows, arterial pressure, and heart rate were continuously recorded on a Beckman Dynograph. We allowed 30 to 60 minutes for stabilization of these parameters before beginning the following experiments.

Experimental Protocols

Groups 1 and 2: Central and Peripheral Infusions of Antihypertensive Polar Renomedullary Lipid (APRL)

Animals in Group 1 had intact baroreceptor reflexes. Group 2 animals were subjected to sinoaortic baroreceptor deafferentation (SAD) according to a modification of the method of Krieger. Briefly, this involved aseptically exposing the right and left carotid sinuses and stripping all connective tissue and nerves from the internal, external, occipital, and thyroid arteries in the carotid bifurcation region. The exposed vessels were painted with 10% phenol in ethanol, with care taken to avoid damaging the vagi and other nearby nerves. The right and left superior laryngeal nerves near the vagi were cut, as were the cervical sympathetic trunks and any distinct aortic depressor nerves that were located. All SAD animals in these studies were allowed 5 to 10 days to recover and regain pre-SAD body weight before being subjected to chronic instrumentation with flow probes and catheters.

On the day of the experiment, SAD animals were first tested for absence of baroreflex control of heart rate. Each rat received i.v. injections of nitroglycerin (120 µg/kg) to decrease arterial pressure by approximately 50 mm Hg and phenylephrine HCl (1 to 3 µg/kg) to increase mean arterial pressure (MAP) by a similar amount. Pre-nitroglycerin and pre-phenylephrine heart rates (HR) averaged 398 ± 11 and 400 ± 12 bpm, respectively. For all studies, only SAD animals exhibiting a change in HR of ≤ 15 bpm were considered adequately debuffered and selected for study. This criterion resulted in rejection of approximately 30% of the animals with SAD.
Graded i.v. doses of APRL were administered to Group 1 (10, 50, 100, 500, 1000 ng) and Group 2 (1, 5, 10, 50, 100 ng) animals in volumes of 5 to 100 μl, each followed by 0.2 ml of saline to flush the venous catheter. The sequence of administration of APRL was randomized for all but the highest dose in each group. Time (15–30 minutes) was allowed between each injection of APRL for reestablishment of control values. The response to intracerebroventricular (i.c.v.) administration of APRL was then evaluated for Groups 1 and 2. APRL (10, 50, 100, 500, 1000 ng) was given in 1 to 5 μl volumes through a 30-gauge injector needle placed in the i.c.v. guide cannula and connected to a 10 μl syringe via a 50 cm PE 10 catheter. The schedule of administration was randomized, and at least 10 minutes was allowed between each dose. Intravenous and i.c.v. administrations of APRL were conducted on different days with several days intervening, and the order of administration was randomized. The vehicle for i.v. and i.c.v. APRL (saline) had no effect on any hemodynamic parameter. In this and in all subsequent protocols, the animals did not receive intravenously more than 1.5 cc of total volume per hour.

**Group 3: Vascular Reactivity**

A third group (n = 6) of chronically instrumented rats with SAD was prepared for determination of the effect of APRL on vascular reactivity to pressor agents. After verification of the adequacy of the SAD procedure, the rats received, in random order, bolus i.v. injections of norepinephrine (50, 150, and 500 ng/kg), angiotensin II (12, 25, and 75 ng/kg), and vasopressin (1, 3, and 10 mU/kg); control conditions were verified as described above. Blood pressure and HR responses to i.c.v. ANRL were determined for dosages (10, 50, and 100 μg; 1, 5, and 10 μl) that were administered in random order, with 15 to 30 minutes allowed between each administration. After central administration, the response to a single intravenous dose (500 μg) was obtained. The vehicle for ANRL (8% ethanol in isotonic saline) was also administered peripherally and centrally in each animal and was without effect.

Norepinephrine bitartrate (Sigma Chemical Company, St. Louis, Missouri) and vasopressin (Pitressin, Parke-Davis, Morris Plains, New Jersey) were prepared in saline. Angiotensin II (CIBA) was prepared for injection from frozen concentrated aliquots that were diluted in saline. All drugs were prepared on the day of the experiment and kept at 4°C in a dark container prior to administration. The APRL and ANRL were extracted from fresh renal medullae of the rabbit, as described previously. Frozen aliquots of APRL and ANRL were dissolved in saline (saline plus 8% vol/vol ethanol for ANRL) via 30-second sonication and kept at 4°C for 4 weeks, over which time no loss in potency was detected.

At the completion of each experiment, the patency of the lateral ventricular cannula was determined by injecting 2 μl of a 1:2 dilution of a saturated solution of pontamine sky blue dye into the ventricular system. The animal was then anesthetized with ether and perfused transcardially with saline followed by 10% buffered formalin. Patency of the cannula was indicated by the presence of the dye in the median eminence region of the hypothalamus.

**Data Analysis**

Maximum responses were determined for each pharmacological intervention and compared to control values for each parameter that were obtained for the 1-minute interval immediately before drug administration. Since the miniaturized Doppler-flow probe, as used in this study, does not allow a measurement of absolute resistance in units of mm Hg/ml/min, relative resistance in each vascular bed was calculated from the formula: MAP/blood velocity (Doppler shift in kHz). Changes in regional vascular resistance were expressed as a percentage of control resistance. This is a valid determination of vascular resistance, since the measured blood velocity is directly and linearly proportional to volume flow measured with electromagnetic flowmeters. This proportionality assumes that the cross-sectional area of the vessel adjacent to the implanted flow probe, the velocity of sound through tissue, and the acoustical and geometrical coupling between the piezoelectric crystal and the vessel remain constant.

Data were subjected to a two-way analysis of variance followed by the Tukey procedure for multiple comparisons. The null hypothesis was rejected at p < 0.05. Group values are expressed as means ± SEM.

**Results**

Bolus intravenous administration of APRL produced dose-dependent decreases in arterial pressure and regional vascular resistance in baroreflex-intact conscious animals (Figures 1 and 2). Arterial pressure averaged 123 ± 3 mm Hg prior to APRL administration. Both the decrease in arterial pressure and regional resistance generally reached nadirs within 10 to 20 seconds after administration, and all parameters returned to control levels after several minutes for the lower doses and within 30 to 40 minutes for the higher (500 and 1000 ng) doses. Heart rate, which averaged 365 ± 15 bpm during control conditions, exhibited a dose-dependent increase with administration of APRL (Figures 1 and 3).
FIGURE 1. Hemodynamic effects of bolus intravenous (i.v.) injections of antihypertensive renomedullary lipids (APRL) in a conscious rat with intact baroreflexes.

FIGURE 2. Hemodynamic effects of antihypertensive renomedullary lipids (APRL) in intact rats (closed circles, solid lines) and sinoaortically deafferented (SAD) rats (open circles, dashed lines). \( \% \Delta \) = change in parameter as a percentage of control. HQ vascular resistance = hindquarter vascular resistance.
To uncover the direct effects of APRL without superimposed baroreflex compensation, these responses were compared to animals with SAD (Figure 2). During control conditions prior to infusion of APRL, average MAP and HR in SAD animals were \(137 \pm 8\) (mm Hg) and \(410 \pm 17\) bpm, respectively. Animals with SAD were considerably more sensitive to the hypotensive and vasodilator actions of APRL and exhibited no tachycardia. In both intact and SAD animals, the mesenteric circulation was slightly more sensitive to APRL and demonstrated a tendency (though not statistically significant) toward a greater reduction in resistance at the lowest dose of APRL (Figure 2).

Administration of APRL directly into the brain via the ventricular route elicited no hemodynamic effects at the same doses that were given peripherally (Figure 3), nor were any behavioral effects noted. Likewise, in SAD animals no effect was produced by central administration of APRL.

Vascular reactivity was evaluated in a second group of SAD animals to eliminate baroreflex compensation for the hypotensive effect of APRL. In SAD animals prior to APRL infusion, MAP averaged \(119 \pm 5\) mm Hg. After 30 minutes of a constant infusion of APRL (46 \pm 4 ng/min), arterial pressure stabilized at \(90 \pm 4\) mm Hg and did not decrease further over the subsequent 60- to 90-minute period of observation. During continuous APRL infusion, pressor and regional vasoconstrictor responses to norepinephrine were markedly attenuated (Figure 4). A similar depressant effect was produced by APRL on vascular reactivity to angiotensin (Figure 5) and vasopressin (Figure 6).

In a separate group of conscious SAD animals, blood pressure and heart rate responses were determined for both central and peripheral administration of ANRL. Central administration of 10, 50 and 100 \(\mu\)g ANRL had no significant effect on blood pressure (\(-1 \pm 1, -3 \pm 6,\) and \(6 \pm 10\) mm Hg, respectively) or heart rate (\(0 \pm 0, -10 \pm 10,\) and \(0 \pm 0\) bpm, respectively). When given intravenously (500 \(\mu\)g), ANRL produced marked decreases in MAP (\(-45 \pm 16\) mm Hg) and HR (\(-50 \pm 16\) bpm) that were rapid in onset (1-4 minutes) and that lasted from 10 to 22 minutes.

**Discussion**

The main goal of this study was to examine the mechanism by which APRL, believed to be released from the kidney in association with ANRL and possibly other renomedullary lipids, lowers arterial pressure. In previous studies,\(^1\)\(^6\)\(^-\)\(^2\)\(^3\) APRL was shown to exert rapid dose-dependent reductions in arterial pressure. In anesthetized rats with 1K1C renal hypertension of 3 months' duration,\(^6\) i.v. APRL produced similar absolute changes in MAP; however, recovery of arterial pressure required more time.\(^6\) In contrast to our results, Prewitt et al.\(^6\) observed little or no reflex tachycardia and suggested that part of the antihypertensive effect might be central in origin. The absence of tachycardia did not appear to result from a direct negative chronotropic effect, since APRL had no effect on the frequency of contraction of isolated atria.\(^6\)
FIGURE 4. Effect of antihypertensive renomedullary lipids (APRL) on norepinephrine reactivity. Control = prior to constant APRL infusion. Abbreviations are the same as in Figure 1; n = 5 to 6.

FIGURE 5. Effect of antihypertensive renomedullary lipids (APRL) on angiotensin II reactivity. Control = prior to constant APRL infusion. Abbreviations are the same as in Figure 1; n = 4 to 6.
conclusion is strengthened by our observation that APRL had no effect on HR in SAD animals.

In our present study, marked dose-dependent tachycardia occurred during intravenous APRL in intact animals, with recovery time course similar to that of MAP. As estimated from the data in Figure 3, a bolus dose of APRL sufficient to reduce MAP by 8 mm Hg would be expected to increase HR by approximately 70 bpm. This was very similar to the reflex tachycardia (+ 65 bpm) produced by an equidepressor dose of the peripherally acting vasodilator nitroglycerin, which we observed in another study using conscious rats. In a recent study, Muirhead and colleagues observed a similar dose-dependent tachycardia response to infused APRL in conscious normotensive rats.

Administration of APRL via the lateral ventricles produced no effect whatsoever, in contrast to identical doses that, when given peripherally, elicited marked hypotension, regional vasodilation, and tachycardia. Complete absence of any central action was also seen in SAD animals, which indicated that baroreflex buffering was not obscuring a subtle CNS action of APRL in intact animals. The lack of action after central administration of very high amounts of APRL provides functional evidence that the agent does not exert effects at central sites to which it might gain access through fenestrated capillary networks of the circumventricular organs. Thus, APRL does not appear to exert any CNS actions on control of HR or vascular resistance. The lack of significant tachycardia during APRL-induced hypotension observed in anesthetized renal hypertensive rats may have resulted from depression in baroreflex control of HR by anesthesia and/or chronic renal hypertension.

Our observation that i.v. APRL produced dose-dependent short-lived reductions in regional vascular resistance is consistent with a direct action on vascular smooth muscle or sympathetic nerve endings. A similar conclusion was reached in studies on the cremaster muscle microvasculature of the rat, in which local superfusion of APRL produced arteriolar dilation and an increase in microvascular blood flow. However, it should also be noted that APRL given in high concentrations may reduce cardiac output by increasing venous compliance, thereby lowering venous pressure and cardiac filling.

In addition to a direct action on vascular smooth muscle, APRL could reduce arterial pressure by interfering with vasoconstriction produced by endogenous vasoconstrictor agents such as norepinephrine, angiotensin, and vasopressin. Plasma levels of these substances are known to be increased in certain forms of hypertension and may contribute to the elevation of peripheral resistance. We tested this hypothesis by examining the effect of continuous APRL infusion on the pressor and regional vasoconstrictor responses to each of these pressor agents in SAD animals. The objective was to see if the baroreflex-induced changes interfered
with peripheral vascular resistance. Elevated levels of circulating APRL uniformly decreased pressor responsiveness and regional vascular reactivity to norepinephrine, angiotensin, and vasopressin. This nonspecific reduction in vascular reactivity to pressor agents produced by APRL was similar to that reported for other vasodilator-antihypertensive agents including hydralazine, diazoxide, nitroprusside, and minoxidil. These agents are thought to have a common mechanism of action that interferes with one or more components of the excitation-contraction coupling mechanism.18

An additional objective of this study was to determine whether the cardiovascular effects of ANRL depend on an action of the lipid on the CNS. Like APRL, ANRL exhibits potent depressor actions when given peripherally.18 However, whereas APRL-induced hypotension results in an increase in HR and efferent renal nerve activity, ANRL-induced hypotension of a similar magnitude is associated with bradycardia and a decrease in renal nerve activity in conscious baroreflex-intact rats.18 Thus, reductions in HR produced by ANRL, unlike those by APRL, are opposite in direction to those expected to occur during hypotensive episodes. Our observation of hypotension and bradycardia following intravenous ANRL in areflexic SAD animals is in agreement with previous findings in baroreflex-intact rats.18 Hence, the presence or absence of baroreflexes does not appear to affect the character of the response to ANRL.

The anomalous negative chronotropic and reduced renal nerve activity responses to intravenous ANRL could be due to a direct action on the CNS. However, the failure in the present study of ANRL, like APRL, to elicit any cardiovascular or behavioral actions when given by the cerebroventricular route argues against this hypothesis. Nevertheless, these results do not rule out the possibility that either APRL or ANRL might exert an indirect action on the CNS through activation of receptor mechanisms accessed within the cerebral vasculature. Selective administration of the lipids to the cerebral vasculature via vertebral or carotid artery infusion would provide one approach to evaluate this possibility. It is also possible that ANRL could act through some other peripheral receptor mechanism, although the present study rules out the possibility that this could involve carotid and aortic baro- and chemoreceptor nerve endings, since the SAD procedure did not alter the response from that seen in other studies18 for baroreflex-intact animals. An additional possible mechanism of action is one by which ANRL and/or APRL undergoes conversion within the plasma to substances that might then exert direct actions on the CNS.

The concept of APRL and ANRL as antihypertensive renomedullary "hormones" has emerged from observations of the protection against prohypertensive interventions afforded to animals with transplanted renomedullary interstitial cells, the cells in which these antihypertensive lipids originate.1-12 Further support for this concept has come from experiments that examined the mechanism behind the rapid normalization of blood pressure following unclipping of the renal artery of 1K1C and 2K1C hypertensive animals.13-18 In these studies, unclipping was associated with degranulation of renal interstitial cells and the release of APRL and ANRL into venous blood from the unclipped kidney, and a decrease in blood pressure, heart rate, and renal nerve activity. These cardiovascular and sympathetic actions can be mimicked by intravenous infusion of venous blood collected from a kidney following unclipping13 or from infusion of ANRL.18 Thus, ANRL appears to be the main antihypertensive hormone released along with APRL under these experimental conditions.

In conclusion, APRL and ANRL, which under certain conditions may be released from the kidney to lower arterial pressure, appear to act peripherally. For APRL, hypotension results from a generalized decrease in vascular resistance and a nonspecific reduction in reactivity to endogenous vasoconstrictor agents. Additional studies are needed to identify, particularly for ANRL, the exact mechanism of action. Furthermore, the pathological conditions under which these substances may prevent hypertension, on the one hand, and under which they remain ineffective in this capacity, on the other, remain to be precisely determined.

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