Antihypertensive Polar and Neutral Renopapillary Lipids

Which is a Hormone?

E. ERIC MUIRHEAD, M.D., LAWRENCE W. BYERS, PH.D., BÖRN FOLKOW, M.D., PH.D., GUNNAR GÖTHBERG, M.D., PETER THOREN, M.D. AND BENNIE BROOKS

SUMMARY Two antihypertensive lipids can be derived from the renal papilla, the antihypertensive polar (APRL) and the antihypertensive neutral (ANRL) renomedullary lipid. The renal venous effluent of the unclipped kidney contains both ANRL and APRL. This effluent lowers the arterial pressure (AP) of the normal rat when infused i.v. As it lowers the AP the heart rate (HR) and sympathetic nerve activity (SNA) are depressed. ANRL infused i.v. also lowers HR and SNA as it depresses the AP. Conversely, APRL elevates HR and SNA as it lowers the AP. Thus, of the two lipids in the renal venous effluent after unclipping, ANRL appears to be dominant. APRL, however, in the renal venous effluent could potentiate the action of ANRL. The net effect of these observations is to support the view that ANRL is an antihypertensive hormone liberated by the kidney after unclipping.

The renomedullary interstitial cells (RIC) degranulate after unclipping. ANRL can be derived from these cells. Thus, the RIC, cells known to exert an endocrine-type antihypertensive function, may well be the source of ANRL in the renal venous effluent after unclipping. The hormonal action of ANRL appears as a major cause of the lowering of the AP after unclipping. It is not known what factors modulate the RIC endocrine system. There is a suggestion that angiotensin may be one of these factors based on the ineffectiveness of these cells toward retarding hypertension when the circulating plasma angiotensin level is high, and their effectiveness when the circulating plasma angiotensin level is low.

(Hypertension 5: V-61-V-65, 1983)

KEY WORDS • renomedullary lipids • antihypertensive lipids • APRL • ANRL • antihypertensive hormone • renomedullary interstitial cells

Two lipids that exert an antihypertensive action have been derived from the renal papilla1 and the renomedullary interstitial cells grown in monolayer cell culture.2 One is highly polar, the antihypertensive polar renomedullary lipid (APRL), and the other is nonpolar or almost so, the antihypertensive neutral renomedullary lipid (ANRL). APRL causes a near sudden depression of the arterial pressure (AP) when injected as a bolus dose i.v. into the hypertensive rat. ANRL, after a lag period of about 2 minutes, causes a slower depression of the AP when injected in the same way. Thus, the two lipids differ biologically.

The question at issue is whether these lipids contribute to the antihypertensive action of the kidney in the manner of a hormone. A corollary to this question is whether one lipid dominates over the other as a naturally occurring antihypertensive substance. We address these questions in this report.

Materials and Methods

The first four steps in the derivation of APRL and ANRL were similar (fig. 1).3-5 These consisted of: 1) homogenization of fresh renal papilla in a buffer; 2) incubation of the homogenate at 37°C for 30 minutes; 3) freeze-drying the slurry; and 4) total lipid extraction (Bligh and Dyer). For APRL generation, the derived oil was treated with: 5) the reducing agent vitride, and then 6) acetylated.3 The next three steps consisted of one-column and two thin-layer chromatographic procedures using different solvent systems. For APRL, the activated silica column (Unisil, Clarkson Chemical Company, Williamsport, Pennsylvania) was washed with chloroform, and then 7) eluted with CHCl3:MeOH:HOAC:H2O (chloroform: methanol:acetic acid: water, 50:25:8:4, vol/vol). The first 8) TLC system consisted of CHCl3:MeOH:HOAC:H2O (50:25:8:3, vol/vol), and the second 9) consisted of CHCl3:MeOH:NH4OH (75:25:4, vol/vol). The APRL used in the present studies was found near the origin in the second system.

For ANRL, the Unisil column was eluted with 5) CHCl3.4 The two TLC systems consisted of CHCl3:MeOH:HOAC:H2O (60:35:8:4, vol/vol) and hex-
### Derivation of APRL and ANRL

1. Renopapillary tissue
2. Homogenized
3. Incubated
4. Freeze-dried
5. Total lipid extracted
6. Vitride reduction
7. Unisil column
8. Acetylation
9. TLC 1, 2, 3, and 4
10. Unisil column
11. TLC 1 and 2

**Figure 1.** Flow chart identifying the major steps in the derivation of APRL and ANRL from fresh renal papilla. APRL is semisynthetic by this approach. ANRL is a natural product.

### Presence of ANRL and APRL in the Renal Venous Effluent after Unclipping the One-Kidney, One Clip Hypertensive Animal

Unclipping the one-kidney, one clip hypertensive rat after ureterocaval anastomosis was attended by the AP reaching normal in an average of 20 hours. After 5 hours, the AP dropped substantially. At this time an infusion was started in the jugular vein, and blood was collected from the renal vein (a total of 50 to 60 ml). The plasma was separated, freeze dried, and subjected to the two TLC dimensions for ANRL and APRL.

### Sympathetic Nerve Activity (SNA), Heart Rate (HR), and Arterial Pressure (AP) after i.v. ANRL and APRL

The techniques of Ricksten and Thoren were used. Under chloralose anesthesia, the left renal pedicle was isolated, the renal nerve was separated from the renal vein and artery. A thin bipolar silver electrode was placed on a segment of the nerve and fixed with silicone rubber. Through a cable, nerve activity was amplified and rectified and recorded on a Grass polygraph. Arterial pressure was measured through the tail artery. A tachogram recorded HR. The animals were under light anesthesia. ANRL was injected i.v. as a bolus dose (100 μg). APRL was infused so as to lower the AP to the same extent as that induced by ANRL. These procedures were conducted on WKY rats.

### Dose Response to ANRL

ANRL has been shown to give a dose response. In the present study, the dose response to ANRL was determined by injecting the compound i.v. as a bolus to hypertensive rats and determining the maximum drop in AP, i.e., the AP at the nadir of the effect. The doses consisted of subthreshold, near threshold and two and four times threshold levels. (Since ANRL is an active principle, i.e., impure, its specific dose cannot be established.)

### Effect of Combining APRL and ANRL

A dose of APRL was injected i.v. After recovery, a dose of ANRL was also injected i.v. After recovery, the same dose of APRL was added to the same dose of ANRL and the mixture was injected i.v. The effect on the AP of the three maneuvers was observed.

### Figure 2

**Upper Panel:** Biologic activity of ANRL. Note the lag period of 2 minutes after the i.v. injection (at the arrow) followed by the decline in AP (from ~ 185 to 135 mm Hg) over 5 minutes. The entire response occurred over 16 minutes. **Lower Panel:** Biologic activity of APRL (dose 0.2 × 10^-6 M). The drop in AP after the i.v. injection (at the arrow) was prompt (from 200 to 95 mm Hg). Recovery occurred in 18 minutes.
Angiotensin and the Antihypertensive Action of RIC

The juxtaglomerular cells (JGC) have been derived in monolayer cell culture. Injected s.c. into a syngeneic recipient followed by reduction of the renal mass, these JGC induced a hypertensive state. The developmental phase of this hypertension (about 3 weeks) was associated with a high circulating plasma angiotensin II/III level. The maintenance phase of this hypertension (after 30 days) was attended by a low plasma angiotensin level.

Cultured renomedullary interstitial cells (RIC) (average 78 million) were transplanted subcutaneously into syngeneic recipients that had received the JGC and had the renal mass reduced, in one group at the time of the JGC transplant (developmental phase of the hypertension) and in a second group after 30 days (maintenance phase of the hypertension).

Results

ANRL Response vs APRL Response

Figure 2 upper panel reveals the characteristic response to ANRL as a bolus i.v. Note the lag period of about 2 minutes followed by a drop of the AP from about 185 to 135 mm Hg over 5 minutes. Recovery of the AP required 12 to 14 minutes.

Figure 2 lower panel relates the APRL activity after the i.v. bolus dose. The drop in AP was prompt (from 200 to 95 mm Hg). Recovery was 90% complete in 18 minutes.

Dose Response to ANRL

As shown in figure 3, there was a threshold dose for the ANRL response. Increasing doses gave corresponding greater depressor effects. Thus, a dose response was demonstrated.

Action of Combined APRL and ANRL

Figure 4 (upper panel) reveals the effect on the AP of a dose of APRL followed by a dose of ANRL. The lower panel reveals the effect of combining the same doses of APRL and ANRL.

The single APRL dose caused an acute depression from 175 to 100 mm Hg. The effect lasted about 6 minutes. The single ANRL dose caused a drop in AP, after a lag period of 2 minutes, from 175 to 155 mm Hg in 6 minutes, a drop that lasted 16 minutes. The combined doses caused an acute effect from 175 to 150 mm Hg and a continued slower decline to 130 mm Hg over 8 minutes. Recovery required 40 minutes. Thus, the two lipids injected together gave a greater depressor effect lasting much longer. This seeming potentiation is under scrutiny.

The presence of both APRL and ANRL in the renal venous effluent after unclipping has been demonstrated. This raises the issue of whether the APRL potentiates the action of ANRL after unclipping, as shown above for the lipids derived from renal papilla.

Figure 5 summarizes the results of i.v. injection of ANRL and APRL on AP, HR, and SNA. The AP was
depressed to the same extent following the two lipids, namely, −35%. ANRL caused a 4% depression of the HR; APRL caused an 8% elevation of the HR. ANRL depressed SNA by 33% while APRL elevated SNA by 32%. It is important to note that, APRL caused tachycardia in the lightly anesthetized animal, presumably reflexly induced, while accentuating sympathetic activity, and ANRL caused no tachycardia and suppressed the sympathetic nervous system. (APRL caused no tachycardia and at times bradycardia in the deeply anesthetized animal.) These were additional indications of the biologic differences between these two lipids.

Transplantation of RIC s.c. at the same time as the transplantation of JGC s.c., but at a different site, followed by uninephrectomy was associated with the development of hypertension in the same way as that following the transplantation of JGC alone (upper panel, fig. 6). It is to be remembered that, during the development of this hypertensive state, the plasma angiotensin II level is high. Conversely, the transplantation of RIC s.c. more than 30 days after the transplantation of the JGC s.c. followed by uninephrectomy, when the hypertensive state was fully developed (maintenance phase) was associated with a significant drop of the AP from an average of 171 to 136 mm Hg (lower panel, fig. 6). During the maintenance phase of this hypertensive state, the plasma angiotensin II level was low.

Thus, there appeared to be an inverse relationship between the level of plasma angiotensin and the antihypertensive action of the RIC. This relationship suggests that angiotensin has a constraining effect on the antihypertensive function of the RIC. Since the antihypertensive function of the RIC appears to be mediated by its hormone, ANRL, angiotensin may modulate the secretion of this hormone within the kidney. This is our present hypothesis.
ANTIHYPERTENSIVE POLAR AND NEUTRAL LIPIDS/Muirhead et al.

Discussion

Unclipping the Goldblatt hypertensive rat (either the one-kidney, one clip or the two-kidney, one clip type) is followed by a rapid decline of the AP to normal levels. Removal of the resistance afforded by the clip contributes in a minor way to the drop in AP. The major mechanism(s) involved in the lowering of the AP following unclipping remains obscure. It is now possible to consider at least one major mechanism involved in the antihypertensive effect.

The renal venous effluent of the unclipped kidney infused i.v. causes a lowering of the AP of a normal animal while at the same time lowering the HR and SNA. This is precisely the same response to ANRL extracted from the renal papilla and injected i.v. into a normal animal (fig. 5). As the AP drops following unclipping, the RICs in the unclipped kidney degranulate. This degranulation coincides with the appearance of ANRL and APRL in the renal venous effluent. Moreover, ANRL can be extracted from RIC grown in monolayer cell culture. It is a natural product since it is derived directly from the renal papilla and the RIC.

The indications are that ANRL is the dominant lipid in the renal venous effluent following unclipping, since the renal venous effluent following unclipping and the ANRL extracted from renal papilla behave in the same way toward the AP, HR and SNA. The APRL in the renal venous effluent following unclipping, however, may potentiate the antihypertensive action of ANRL.

These various observations are consistent with ANRL being an antihypertensive hormone secreted by the RIC following unclipping. The action(s) of this hormone seems to contribute in a major way to the antihypertensive effect following unclipping.

APRL contains the platelet-activating factor (PAF). It is not a good candidate for a renal antihypertensive hormone since it aggregates platelets, liberates leukotrienes, and increases HR and elevates sympathetic tone. ANRL, on the other hand, appears as an ideal antihypertensive substance as it lowers HR (or at least does not cause tachycardia) and suppresses sympathetic tone as it lowers the AP.

There is little doubt that the RIC exert an antihypertensive action by secreting an antihypertensive hormone. It is not known what factors regulate the secretion of this hormone. As indicated by the early and late transplantation of RIC in the JGC-induced hypertensive rat, angiotensin may have a constraining effect on the secretion of this hormone. This hypothesis becomes more attractive when one considers the likelihood that the JGC secrete angiotensin directly. Such secretion by the juxtamedullary juxtaglomerular complex could modulate the secretion of the antihypertensive hormone (ANRL) by the RIC within the kidney. In this context it is of interest that unclipping suppresses the renin-angiotensin system.

Conclusion

ANRL appears to be the antihypertensive hormone of the renomedullary interstitial cells.

References

Antihypertensive polar and neutral renopapillary lipids. Which is a hormone?
E E Muirhead, L W Byers, B Folkow, G Göthberg, P Thorén and B Brooks

Hypertension. 1983;5:V61
doi: 10.1161/01.HYP.5.6_Pt_3.V61

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/5/6_Pt_3/V61

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/