The Liver Converts the Antihypertensive Hormone of the Kidney
The Renohepatic Axis

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SUMMARY The antihypertensive neutral renomedullary lipid, derived from the renal papilla, causes a vasodepressor effect when injected into a peripheral vein, such as the inferior vena cava, after a lag period of 1 to 2 minutes. The blood pressure tracing is skewed (cuplike effect). The lag period is significantly reduced after injection of the antihypertensive lipid into the portal vein. The vasodepressor configuration (cuplike) is the same whether the lipid is injected into the vena cava or portal vein. Removal of the liver from the circulation prevents the depressor effect. Thus, passage through the liver is essential for antihypertensive lipid activity. Renoportal shunting of blood potentiates the antihypertensive function of the kidney after unclipping in the one-kidney, one clip hypertensive rat. Lack of a hepatic circulation prevents the antihypertensive function of the kidney after unclipping. Antihypertensive neutral renomedullary lipid and the renal venous effluent after unclipping have the same biological behavior. We conclude that antihypertensive neutral renomedullary lipid is a promolecule, the putative prohormone of the renal papilla and its renomedullary interstitial cells. The liver converts the antihypertensive prohormone of the kidney to an active antihypertensive substance. A renohepatic axis of blood pressure control appears to exist.

KEY WORDS • antihypertensive neutral renomedullary lipid • hepatic conversion • renoportal shunt • unclipping • renohepatic axis

The antihypertensive neutral renomedullary lipid (ANRL) is the putative antihypertensive hormone of the kidney and its renal papilla. Extracted from the renal papilla and purified, it usually induces a blood pressure response after a bolus injection into a peripheral vein of a hypertensive rat. There is a lag period of 1 to 2 minutes followed by a relatively slow decline of the blood pressure to its nadir. Thus, the pressure tracing is skewed, giving the configuration a cuplike appearance (see Results). This effect is dose-dependent. The characteristic lag period of ANRL suggests that it is converted to an active molecule somewhere in the body. The proposed conversion does not occur in the heart and lungs, as the material is injected directly into these areas. It does not occur in the kidneys, as the usual lag-depression effect takes place after bilateral nephrectomy (E.E. Muirhead and B. Brooks, unpublished observations, 1985). It does not occur in the peripheral microcirculation, as ANRL does not dilate vessels of the isolated lower extremity of the rat. We therefore decided to test the effect of ANRL when injected directly into the portal vein. The results of these experiments, as well as others related to them, are herein reported. These experiments not only indicate that ANRL is a putative prohormone but emphasize a relationship between the kidney and the liver in regard to blood pressure control.

Materials and Methods

Male Wistar rats were used (from Harlan Sprague-Dawley, Indianapolis, IN, USA). They were about 7 weeks old upon arrival and weighed 200 to 225 g. The methods used to produce one-kidney, one clip hypertension and to introduce aortic (lower abdominal aorta) and venous (lower inferior vena cava) catheters have been described in detail. Both catheters were brought out at the back of the neck. The rats were

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studied 2 to 5 months (average, 3.43 months) after the renal manipulations (weight, 400-500 g) when the hypertension was fixed in its maintenance phase (blood pressure, 160-200 mm Hg).

ANRL was extracted and purified from fresh rabbit renal papilla, as previously described, except that two additional thin-layer chromatographic procedures were performed: ether/acetic acid/water (100:0.5:0.5) followed by acetonitrile/methanol/85% phosphoric acid (130:5:1.5). Elution from the Kieselguhr G plate was performed as previously described.

ANRL is a nonpolymer and must be emulsified before it can be injected. ANRL from the chromatographic fourth dimension (0.5-1.0 mg) was at the bottom of a 10 x 75-mm Pyrex test tube, in the form of a film. It was removed from the tube and placed in 0.5 ml of 2% rat albumin (Fraction V; Miles Laboratory, Naperville, IL, USA) in saline by sonication in a water sonicator (Branson Sonagener Model D-5. Branson Sonic Power, Danbury, CT, USA) at 37°C for 10 to 15 seconds. The sample was cooled under tap water and then further sonicated at room temperature for 10 to 15 seconds by a probe sonicator (Heat Systems Ultrasonic Cell Disruption Model W185F, Heat Systems, Farmingdale, NY, USA) equipped with a special stepdown microtip. It became a smooth emulsion (no particles) and could be easily injected intravenously without a residue. Unless ANRL forms a smooth emulsion, it is inactive.

The animals in all groups (experimental and control) were subjected to pentobarbital anesthesia (30 mg/kg i.p.). With rare exceptions, this was the only dose of anesthesia.

Blood pressure was measured the week before the experiment on at least 3 days, by the tail-cuff method (E and M physiograph assembly, E and M Company, Houston, TX, USA), to confirm that the animal was hypertensive. For 30 minutes before and continuously throughout each experiment, the blood pressure was measured directly from the abdominal aorta by means of a Statham P23Dc transducer (Oxnard, CA, USA) and a Grass Model 7 polygraph (Quincy, MA, USA). For the animal to qualify for a given experiment, the 30-minute blood pressure and the pressure recorded the week before had to be comparable. The difference between these pressures was on the order of 5 mm Hg.

In all experiments a catheter (PE10) was inserted into the lower portion of the remaining ureter to collect urine before and during the experiments. The urine samples were frozen and analyzed later.

Statistical analysis was by Student's t test.

Experimental Groups

In Group 1 ANRL was injected into the inferior vena cava (n = 17) and into the portal vein (n = 19), according to the following procedure. An abdominal incision was made, and the portal vein was exposed for injection. ANRL was first injected as a bolus into the caval catheter. In 11 experiments, after the response and recovery over a period of 16 to 72 minutes (average, 41 minutes), the same dose of ANRL was injected into the portal vein of the same rat. In the remainder of the experiments different rats were used for the caval (n = 6) and portal (n = 8) injections. The results in the two groups were the same, and they were combined. On each occasion a sterile, plastic tuberculin syringe (1.0 ml) and a 27-gauge needle were used.

In Group 2 ANRL was injected into the vena cava or portal vein (or both), the liver was isolated from the circulation, and a repeat injection was made into the vena cava (n = 4). The rationale for this procedure was to determine whether circulation through the liver was essential for the depressor effect of ANRL. The protocol for these experiments was as follows. Under pentobarbital anesthesia, an abdominal incision was made, and the cannulae were inserted into the abdominal aorta and inferior vena cava. The cannulae were led subcutaneously to an exit through the skin at the back of the neck. ANRL was injected into the vena cava or portal vein (or both). After recovery, the animal was heparinized (0.05 ml i.v., 50,000 U of Upjohn heparin). A flexible catheter (Silastic tubing; inside diameter, 0.157 cm, outside diameter, 0.241 cm, with each end having a 2.54-cm PE240 beveled tip with a ridge to secure it in place with a tie) was filled with saline and introduced into the right jugular vein pointing proximally and then into the portal vein pointing distally (portojugular shunt). Circulation was immediately established between the splanchnic (portal) bed and the jugular vein. The proximal segment of the portal vein was ligated. The hepatic artery was isolated and ligated. Thus, circulation to the liver was stopped. ANRL (same dose) was then injected into the inferior vena cava.

In Group 3 a renoportal venous shunt was performed, plus unclipping of the Goldblatt kidney (n = 9). After pentobarbital anesthesia had been administered, the lower abdominal aorta was cannulated. There were two subgroups. In Subgroup A (n = 6) the arteries to the splanchnic area were ligated. This included the superior mesenteric, caudal, and celiac axis, except for the hepatic artery, which maintained circulation to the liver. This was demonstrated by the flow of arterial blood out of the severed (proximal) portal vein. The gut between the rectum and duodenum was removed. The animal was heparinized (0.05 ml containing 50,000 U of heparin i.v.), and a blood transfusion containing an additional 50,000 U of heparin was given intravenously (volume, 3 to 9 ml of whole blood; average, 5.6 ml). A shunt with a loop was established between the renal vein and the portal vein by means of a Silastic tube (same as that used for the portojugular shunt). Blood immediately flowed between the renal and portal veins. The clip on the renal artery was then removed, as previously described. The blood pressure dropped during the surgical manipulations but returned to prior hypertensive levels after the blood transfusion (or transfusions).

In Subgroup B (n = 3) the arteries were not ligated. Instead, a portojugular shunt was established (splanchnic area intact), followed by a renoportal shunt and removal of the clip. This subgroup acted as a control for the effect of the removal of the gut.

In Group 4 isolation of the liver from the circulation
was followed by unclipping ($n = 6$). The rationale for this approach was to determine whether the liver with an intact circulation was essential for the antihypertensive function of the kidney to occur after unclipping. The liver was isolated from the circulation, as described above, and the renal artery clip was removed.

**Controls**

Controls for Groups 1 and 2 received an injection of ANRL into the vena cava (Group 1) and the portal vein (Group 2); they represented intrinsic controls.

For Group 3 there were two sets of controls. In one unclipping occurred with the gut left in ($n = 3$) or removed ($n = 3$), in both cases without the renoporal shunt. There was no difference between these subgroups, and they were combined. In the second set of controls for Group 3 a renoporal shunt was performed with the gut removed but without unclipping ($n = 6$).

The controls for Group 4 underwent unclipping without isolation of the liver, the same as for Group 3.

**Results**

The characteristic response of ANRL is shown in Figure 1.

**Group 1 (Caval vs Portal Injection)**

Injection of ANRL into the inferior vena cava (0.5–1.0 mg in 0.05 ml as a bolus; Figure 2) was followed by the cuplike depression in pressure after a lag of 1 minute. The same dose given in the portal vein of the same rat resulted in a rapid drop in blood pressure followed by the same cuplike depression. Thus, in this example, as well as others (Table 1), the configuration of the depressor effect was maintained; only the lag period was lost.

Table 1 presents the group results. The hypertensive blood pressure of the portal group was higher than that of the caval group, mainly because of a pressure rebound in those animals receiving both caval and portal injections. There was no significant difference in the drop in blood pressure, the time to reach the nadir, or the duration of the depressor effect after the caval and portal injections. This indicated, as shown in Figure 2, that the same cuplike depression occurred after injection at the two sites. The main difference between the responses was in the lag period (1.95 ± 0.2 minutes vs 0.44 ± 0.2 minutes, $p < 0.001$). In 10 of 19 portal responses there was virtually no lag period, meaning only a few seconds and unmeasurable by the blood pressure tracing. Similar results were observed after a fourfold increase in the speed of the blood pressure tracing (caval injection, 112 ± 7.5 seconds; portal injection, 57.9 ± 10.5 seconds; $n = 4$; $p < 0.01$).

The results indicate that the effect of ANRL when injected into the liver is modified by the almost complete absence or major decrement of the lag period that is associated with injection into a vein going directly to the heart and lungs.

**Group 2 (Isolation of the Liver)**

When the liver was isolated from the circulation (portojugular bypass plus hepatic artery ligation), ANRL failed to cause the depressor effect. In the single example shown in Figure 3, a very potent ANRL preparation was used. Yet, when this same batch was...
given in the same dose to the same animal after the liver had been removed from the circulation, no depressor effect was observed. Two animals had ANRL injected into the vena cava (blood pressure, 180 and 175 mm Hg; lag period, 2.0 and 2.2 minutes; blood pressure depression, −155 and −80 mm Hg), followed by injection into the portal vein (blood pressure, 185 and 185 mm Hg; lag period, 0.6 and 0.6 minutes; blood pressure depression, −100 and −50 mm Hg), and finally, injection into the vena cava with the liver out of the circulation (no change in pressure). The four animals had equal blood pressure before portal injection (liver intact) and caval injection (liver excluded), but in the former case the pressure dropped (− 83 ± 21 mm Hg), and in the latter there was virtually no change in pressure. Thus, circulation through the liver appears to be essential for ANRL to cause its vasodepressor effect.

Group 3 (Renoportal Shunt plus Unclipping)

Figure 4 summarizes the results with this group when the gut was removed before the shunt. Within 10 minutes after unclipping with the shunt in place, the blood pressure dropped significantly (from 177 ± 5 to 143 ± 11 mm Hg, p < 0.01) and continued to drop, reaching 129 ± 10 mm Hg after 20 minutes (p < 0.001) and a low normal value of 103 ± 7 mm Hg after 30 minutes (p < 0.001). This depressor effect was still observed after about 90 minutes (88 ± 9 mm Hg, p < 0.001). The results were further analyzed by examining the drop in blood pressure from hypertensive levels to levels after unclipping in terms of the decrement in pressure per minute, as previously described. At 30 minutes, the change in pressure per minute was 2.46 ± 0.19 mm Hg in the shunt group and 0.36 ± 0.2 mm Hg in the controls (p < 0.001). The results with the gut in (portojugular and renoportal shunts with unclipping, n = 3) were the same as those with the gut out (blood pressure, 173–153, 135, and 107 mm Hg in 10, 20, and 30 minutes, respectively, after unclipping). The pressure in controls remained elevated through the first 30 minutes, dipping slightly by about 90 minutes. Five of the six controls had a normal pressure (115 mm Hg) in an average of 2.9 hours. The other controls for these experiments (renoportal shunt without unclipping, Figure 5) had no significant change in blood pressure over the 3 hours of observation (hypertensive pressure, 191 ± 5 mm Hg; at 10 minutes, 192 ± 4 mm Hg; at 20 minutes, 188 ± 6 mm Hg; at 30 minutes, 188 ± 6 mm Hg; at 90 minutes, 184 ± 6 mm Hg; and at 180 minutes, 186 ± 7 mm Hg). Thus, the renoportal shunt potentiated, in a major way, the antihypertensive function of the kidney after unclipping.

Group 4 (Isolated Liver plus Unclipping)

Removal of the liver from the circulation prevented the drop in blood pressure after unclipping (see Figure 5). Thus, the liver appeared to be essential for the kidney to exert its antihypertensive function after unclipping.

Discussion

The liver markedly reduced the lag period after the bolus injection of ANRL into the vena cava (Group 1). Three hypotheses can be entertained as explanations for this phenomenon. The liver may convert ANRL to an active molecule, the liver may be stimulated to secrete a vasodilator, or ANRL may inhibit the production of a pressor substance by the liver. The conversion hypothesis appears to be the most attractive of the three, since the effect is very rapid, and the end result, whether the injection is made into the vena cava or the
portal vein, is the same (i.e., the skewed, cuplike vasodepressor effect). Also, the hepatic effect is not due to ANRL causing the pooling of blood in the liver and a sudden decrease in cardiac output, as indicated by the fact that the blood-drained, isolated liver gives rise to the same nearly immediate effect when ANRL is injected into it followed by its being flushed by saline into the hypertensive recipient (E.E. Muirhead and B. Brooks, unpublished observations, 1985). If ANRL is modified by the liver, then the question is: Which cells in the liver modify it — endothelium, von Kupffer cells, or hepatocytes? These possibilities can now be subjected to objective experimentation. The hepatocytes remain eligible, since the circulation through the liver is an open one. The liver sinusoids are fenestrated so that plasma (not erythrocytes) can enter the space of Disse and bathe the hepatocytes before returning to the general circulation.

The important role of the liver in the proposed conversion of ANRL is demonstrated by the results in Group 2. When the liver is isolated from the circulation, ANRL is inactive. This role is further supported

**Figure 4.** Results in Group 3. Unclipping the one-kidney, one clip hypertensive rat when the renoportal shunt was in place caused a pronounced increase in the antihypertensive action of the kidney (●●●), as compared with unclipping in the absence of the shunt (○○○).
by the results in Group 4. Isolation of the liver from the circulation prevents the antihypertensive action of the kidney after unclipping. It is already known that unclipping lowers the blood pressure, because the kidney secretes an antihypertensive hormone. Thus, this hormone needs to be acted on by the liver to be effective.

Finally, our observation that the renoportal shunt potentiates the antihypertensive action of the kidney after unclipping (Group 3) adds credence to the concept of a renohepatic axis. The interpretation is that a major bolus of the antihypertensive hormone is delivered to the liver, before its degradation, by this means. This interpretation is supported by the fact that the renoportal shunt is ineffective in the absence of unclipping.

These observations provide further support for the view that ANRL is the antihypertensive prohormone of the renal papilla and its renomedullary interstitial cells. After unclipping, ANRL and the renal venous effluent continue to reproduce each other in terms of activity and their relationship to the liver (present experiments).

The four sets of observations herein presented demonstrate, collectively, that there is a relationship between the antihypertensive hormone of the renal papilla, as demonstrated by multiple experiments, and the liver. The evidence supports the view that the renomedullary interstitial cells secrete this hormone. A renohepatic axis of blood pressure control appears to have been demonstrated.

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