Antihypertensive Functions of the Kidney

Arthur C. Corcoran Memorial Lecture

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KEY WORDS • prohypertensive • antihypertensive • renal medulla • glyceryl ether • renomedullary interstitial cells • extracts • neutral lipid (ANRL) • polar lipid (APRL) •

I AM grateful to the Council for High Blood Pressure Research and to its Program Committee for the invitation to deliver the Third Arthur C. Corcoran Memorial Lecture. I have felt this responsibility keenly since Dr. Bumpus first approached me: first, because, after 16 years as a member of this body, I am well aware of the critical nature of my peers on the Council; and second, because of the immensity of the topic I propose to consider with you — the antihypertensive functions of the kidney — a subject analyzed in depth by many of you here today.

Before beginning, I wish to pay my respects to Dr. Corcoran. It was my pleasure to know him while he was in Cleveland, and especially during his short stay in Ann Arbor — “Core,” we called him. His dedication to the advancement of knowledge in the field of hypertension will remain an example for all of us to follow.

Among my vignettes of Core, I am especially fond of one. As a budding “renoprivinologist,” I was on a program of the research section of the American Medical Association in the early 1950s with Corcoran and others. I delivered a talk on the vascular lesions of renoprival hypertension of the dog. The paper, which appeared in the Archives of Internal Medicine, could be considered respectable, but the talk needed much improvement. Core discussed the talk with praise. He was, indeed, a considerate human being as well as a fine scientist.

The concept of an antihypertensive function of the kidney may be considered, in modern terms, as originating with the Goldblatt experiment, that is, in 1934.* Fortunately, Goldblatt's experimental animal was the dog. In the dog, as he and his associates showed, constriction of one renal artery evoked a transient elevation of the arterial pressure while constriction of both renal arteries induced a permanent hypertensive state. (These relationships are true for the usual degree of renal artery constriction; it is now known that a precise, extreme constriction of one renal artery of the dog will evoke sustained hypertension.) More important for present purposes was the demonstration by Goldblatt, as pointed out later in a Harvey Lecture, that constriction of one renal artery of the dog plus contralateral nephrectomy also created a lasting hypertensive state.

All of us have benefited from the many by-products of these experimental maneuvers. Two early ones may be cited at this time: 1) a revival of interest in the renal pressor agent called “renin” by Tigerstedt and Bergman, and 2) the concept of “a protective action of the normal kidney toward hypertension,” a proposal by workers of the Argentine school, mainly Fasciolo, resulting from their confirmation of Goldblatt’s findings. Thus, a dual role for the kidney in hypertension began evolving soon after Goldblatt, what Braun-Menéndez later designated as “the prohypertensive and antihypertensive actions of the kidney.”

Concepts of the prohypertensive actions of the kidney, albeit troubled by disagreements for many years, were eventually evaluated in such a manner as to lead to the consensus currently in vogue. This began with the discovery of the renin-angiotensin system by the Page* groups, and the Braun-Menéndez* groups, and was followed by establishment of salt retention and the renal actions of mineralocorticoids (Simpson et al. and others) as hypertension-inducing mechanisms. At present, prohypertensive actions of the kidney are proposed by a majority to result from the influence of renal vasoconstrictor agents, mainly angiotensin II, and salt-volume retention due to damaged kidneys, absent kidneys, or the excessive action of mineralocorticoids, mainly aldosterone. (Renal pressor agents other than aldosterone...
proposed but, at present, these are in need of confirmation, more precise characterization, and indications of biologic status. Among these are Grollman and Krishnamurty's nephrotensin,\(^19\) Risler and Fasciolo's corticotensin,\(^19\) Skegg et al.'s renopresin\(^2\) and Bouchert et al.'s tonin.\(^2\) Collectively, these considerations have been designated as either the vasoconstriction-volume hypothesis;\(^5\) or simply as angiotensin and sodium-volume dependence of the hypertensive state.\(^4\) \(^2\)

Before leaving this subject, a word is perhaps in order about renal vasoconstriction. It would seem that mechanisms inducing renal vasoconstriction tend to be prohypertensive.\(^4\)\(^9\) The constriction may result from an extrarenal source, such as the adrenergic nervous system or circulating pressor agents (angiotensin, norepinephrine, etc.); or by an intrarenal effect, such as that of local angiotensin or failure of the countervailing actions of intrarenal dilator agents such as prostaglandins and kinins. The latter matters have been admirably investigated and discussed by McGiff and associates.\(^3\) \(^0\)\(^-\)\(^1\)

If we accept renal vasoconstriction as a prohypertensive phenomenon, it follows that those forces that oppose or relieve such constriction are antihypertensive. This has recently been emphasized by Hollenberg et al.\(^4\)

Concepts related to the antihypertensive actions of the kidney have had great difficulty obtaining acceptance. It appears today that a majority of workers in this field consider the removal of Na-volume loads by diuresis-natriuresis, through either the natural excretory process or induced by diuretic agents, as the main, and possibly the only, antihypertensive function of the kidney. Clearly, this represents a major antihypertensive renal action, although the details of the hypertension-inducing effects of Na and volume have as yet to be sufficiently elucidated. (It has been proposed that a renin inhibitor contributes to the antihypertensive renal action.\(^2\)\(^4\)\(^1\)) Certain additional proposals are mainly of historical interest. Among these were they hypothetical antihypertensive factors sought after in renal extracts by Page et al.,\(^5\)\(^6\) Grollman et al.\(^7\)\(^-\)\(^9\) and others.\(^10\)\(^-\)\(^1\) \(^1\) "Renotropin" was invoked by Braun-Menéndez\(^7\) as a hypothetical, extrarenal pressor protein metabolized by the kidney, and evoking hypertension in the absence of kidneys or when the kidneys were damaged and unable to metabolize it.)

More recently, an antihypertensive function of the kidney unrelated to its ability to regulate electrolyte and water balance has received support from multiple quarters. This relates to what Grollman and Rule\(^4\) conceived of as "a non-excretory antihypertensive function of the kidney." "Incretory," they called this, meaning that the kidney secretes an antihypertensive hormone. In expounding Grollman's view, we developed data in support of performance of this function by the renal medulla and its renomedullary interstitial cells (RIC). This resulted from a sequential study.

### Sequential Study

The design of the sequential study is shown in figure 1. First, renoprival hypertension of the dog was made reproducible in an accelerated fashion, i.e., "standardized" by either a prescribed salt-water-dietary protein intake or by Na-volume loading alone (fig. 2).\(^2\)\(^8\)\(^-\)\(^1\) Next, it was demonstrated that this type of hypertension was reversed by whole kidney transplants despite the maintenance of the Na-volume overload (figs. 3 and 4).\(^4\)\(^8\) This experiment was comparable to that of Kolff and Page\(^4\) using renal perfusion. The results of this experiment were also reinforced by Gómez and associates\(^4\) and Tobian and associates\(^4\) using shorter intervals of renal perfusion in other hypertensive settings involving the rat. Next, it was determined that uretero-caval anatomosis (UCA) prevented this type of hypertension, while ureteral ligation (UL) failed to do so (fig. 5).\(^5\)\(^0\)\(^-\)\(^1\) (UCA had been demonstrated earlier to be protective toward hypertension by Grollman et al.\(^4\) This was supported by Kolff et al.\(^4\) A significant morphologic difference was noted between the latter two preparations. UCA was attended by an intact and enlarging renal medulla while UL was associated with ischemic-type papillary necrosis. (We\(^4\) were prepared to encounter the papillary necrosis in this setting because we had previously described the obstructive type of renal papillary necrosis in the dog.) This functional-morphologic difference between the

![Flow chart showing components of the sequential study used to investigate an endocrine-type antihypertensive function of the renal papilla and its RIC.](https://hyper.ahajournals.org/doi/10.1161/01.HYP.10.4.445)
FIGURE 2. "Accelerated" canine renoprival hypertension. Experimental data used in the development of an "assay" for the non-excretory antihypertensive functions of the kidney. Left: After renal ablation and then dialysis, renoprival hypertension evolved under two conditions: a high protein-low saline and a high saline-low protein input. Right: A mixture of saline-dietary protein, without dialysis, gave the same results over 4 days as when the high protein-low saline dialysis was used. The latter was termed "accelerated" renoprival hypertension. This became the "assay procedure." The hypothesis considered that renal maneuvers that reversed or prevented this hypertensive state were antihypertensive (modified from material in refs. 43-45).

FIGURE 3. Antihypertensive action of whole kidney transplants on renoprival hypertension in dogs. With the dogs on a regimen of dietary protein plus dialysis, the kidney was transplanted to the neck (renal artery anastomosed to carotid artery, and the renal vein to the jugular vein, while the ureter exited at the skin). The dialysis was continued and the estimated urinary fluid loss was replaced as a balanced salt solution. Left: Within 24 hours, the MAP was lowered from over 160 mm Hg to near control level. As this antihypertensive action occurred, there was no weight loss (hatched columns). Right: Sham-operated controls had no change in pressure. CBP = control blood pressure; HBP = hypertensive BP, before transplant (modified from ref. 46).
FIGURE 4. Animals having renoprival hypertension were deliberately fluid-loaded (load 7.9 ± 0.9 liters) and then underwent kidney transplant. Left: The BP was lowered even though the fluid load was maintained — as supported by no weight change. Right: As the arterial pressure was lowered, there was no change in blood and ECF volumes (modified from ref. 46). Reduction of the arterial pressure seemed due not to loss of water and sodium from the animal but, rather, to a non-excretory antihypertensive renal function.

FIGURE 5. Left: Ureterovenous anastomosis plus contralateral nephrectomy protected against accelerated renoprival hypertension resulting from the two approaches (low saline-protein and high saline input). Right: Ureteral ligation (bilateral or unilateral plus contralateral nephrectomy) did not so protect (modified from refs. 50 and 51).
UCA and UL models led us to conceive of the renal medulla (later its papilla) as the source of the antihypertensive action of the kidney under these circumstances.

We then utilized a technique dating back to 1848, namely, autotransplantation of a structure to demonstrate its endocrine nature. Autotransplants of fragmented renal medulla prevented the accelerated renoprival hypertensive state while similar transplants of renal cortex and other tissues did not prevent the hypertension (fig. 6). Renomedullary transplants were then used in a variety of hypertensive models (fig. 6) and were shown to be antihypertensive in all of these by either preventing or reversing the hypertension.

At the same time, it was shown that similar transplants of renal cortex and other tissues did not exert an antihypertensive action. With the guidance of renomedullary tissue, the arterial pressure of one-kidney, one clip hypertensive rats was lowered. When the transplants were removed 14 days later, the pressure returned to prior hypertensive levels. Renocortical transplants were ineffective. There was not only a significant antihypertensive action but a "make and break effect" was demonstrated - when the tissue was in, an action occurred; when the tissue was out, this action disappeared. C. Reproducible model of malignant hypertension was used: a narrow clip was applied to the left renal artery and the right kidney was removed. An early rise in MAP (likely renin-induced) was followed by a near plateau for about 10 days; then a rise to lethal levels occurred during the third week. These animals developed renal insufficiency, widespread fibrinoid necrosis of arteries and arterioles, and encephalopathy. The Tr Med did not prevent the early rise of the MAP, but did prevent the lethal elevation of the malignant phase. Renocortical transplants were ineffective. Moreover, after 3 weeks of protection, the Tr Med was removed, and the MAP elevated rapidly. The animal died in the same manner as controls. The "make and break effect" was once more demonstrated.

Having established the highest sodium load—short of pulmonary edema—tolerated by renoprival rabbits, we found that this regimen of 9 mEq NaCl/kg/day for 4 days, in animals with bilateral nephrectomy regardless of whether they were transplanted with cortical tissue, resulted in an elevation of MAP from 66 to 100 mm Hg. D. The Tr Med, on the other hand blunted the elevation of pressure for 3 days, and on the fourth day, reversed it to near baseline. We concluded: renoprival hypertension, in this experimental model, did not appear to be due solely to hemodynamic changes attendant on the sodium overload. Rather, the hypertensive sequel, which apparently resulted from hemodynamic and possibly other changes attendant upon sodium excess is permitted by the absence of renomedullary factors. To us, the experiments of C and D indicate that a very powerful antihypertensive force exists in renomedullary tissue (modified from refs. 59-62).
given us by Muehrcke and associates, and Tobian and associates, we found that the renomedullary transplants, after 1 to 3 weeks, consisted mostly of RIC and capillaries (figs. 7 and 8). The RIC in these transplants were grown in tissue culture, and, when retransplanted into hypertensive recipients (syngeneic source and syngeneic recipients), exerted the same antihypertensive action as the renomedullary transplants from which they were derived (figs. 9 and 10). The antihypertensive action of the RIC within the renomedullary transplants and by themselves as retransplants is difficult to explain other than by the secretion of a substance(s) that circulates, an antihypertensive hormone.

An endocrine-type antihypertensive action of RIC has been confirmed by nine different groups of workers in eight separate laboratories utilizing the renomedullary transplant approach. Data from four of these laboratories is summarized in figure 11 in which both the reversion and the prevention of the hypertensive state were demonstrated. Confirmation of the antihypertensive action of retransplanted cultured RIC has occurred in one laboratory (Du Charme, personal communication).

Two recent experiments lend additional insight into the endocrine-type antihypertensive function of the renal papilla and its RIC.

Unclipping One-kidney, One Clip Rat at 6–12 Months

The Byrom-Dodson experiment, that of unclipping the one-kidney, one clip hypertensive rat of 6–12 months' duration, has been repeated by several workers for various purposes. Our approach differed from that of others by infusing saline loads intravenously and using three procedures in the same experimental design, namely, free urine flow, ureterocalanastomosis (UCA), and ureteral ligation (UL) in association with the unclipping maneuver. The MAP was recorded continuously after the unclipping. These results are summarized in figure 12.

Unclipping plus the free flow of urine lowered the mean arterial pressure (MAP) to normal within 3 hours. At the same time, there was a brisk diuresis-natriuresis (urine flow 6X, Na excretion 8X controls). This confirmed the results of Ledingham and Cohen, and Liard and Peters. UCA plus unclipping was followed by a normal MAP within about 20 hours. This was comparable to the results of Floyer. A saline infusion was commenced, the clip was removed, and the infusion was maintained at a level equal to or greater than the urine loss, i.e., a positive Na balance was maintained. Four animals gained weight, and three had no weight change; collectively, there was no significant change in weight for the group. The MAP receded to normal in about 20 hours, just as it did following the UCA. These results reinforced those of Ledingham and Cohen, and Neubig and Hooibl. These investigators collected...
Figure 9. Reversion of angiotensin-salt hypertension in rats by transplants of cultured RIC (Tr TCr'ic) is shown in A, B, and D. There were 25 to 30 million cells transplanted subcutaneously. In A, the antihypertensive action remained for the 8-10 days of observation. In B, the antihypertensive action was evident within 6 hours after the cells were introduced (this occurs occasionally). In D, return of the hypertensive state after removal of the Tr TCr'ic is shown. C represents results of the control group receiving either another cell line or preculture media. (From Muirhead, EE, Leach, BE, Pitcock, JA, Germain, GS, Byers, LW, Armstrong, FB, and Brown P: The antihypertensive action of renomedullary interstitial cells grown in tissue culture. Acta physiol. latinoam. 24: 543-549, 1974).

Figure 10. Transplants of cultured RIC are also effective in Goldblatt hypertension. In the two-kidney, one clip model, the maximal effect required about 10 days, while in the one-kidney, one clip model the effect was more precipitous. This antihypertensive action remained for about 30 days, then gradually dissipated. As the effect was lost, so were the transplanted RIC (modified from ref. 69).
Figure 11. Confirmation of the antihypertensive function of the RIC by four different groups is shown. The state of the arterial pressure only was considered. The data of Tobian, of Manthorpe, and of Solez dealt with reversion of the hypertensive state. In 65 examples, the average arterial pressure changed from about 195 to 163 mm Hg, while in 49 control observations there was no change in pressure. The data of Susie dealt with prevention of hypertension in a Na-volume loaded model [congenital hydronephrosis with papillary destruction plus salt] and SHR. The clear bars represent the animals receiving the renomedullary transplants and the hatched bars the controls. B = before transplant; D = during transplant (modified from refs. 72-78).

Figure 12. Observations on unclipping the one-kidney, one clip hypertensive rat are shown. The open circles represent sham-operated animals, the solid circles and squares represent the unclipped animals. A = Unclipping plus freeflow of urine; B = UCA plus unclipping; C = Saline infusion, unclipping, free urine flow and positive Na-balance; D = UCA, saline load plus unclipping; E = Ureteral ligation, saline load plus unclipping; see text for description of results (modified from ref. 91).
the urine following unclipping and returned it to the body intravenously, thereby preventing loss of body fluids. Despite this maneuver, the arterial pressure receded to normal. UCA was instituted, a saline load was added (equal to 1.25%-5.0% body weight), and then the clip was removed. The MAP reached normal in 45-50 hours. Sham-operated controls had no change in MAP. These experiments, as previously considered by different workers, suggest that the unclipping procedure allowed the kidney to exert an antihypertensive action.

Diuresis-natriuresis appeared to accelerate the antihypertensive action of the kidney following unclipping but was not essential for its occurrence. It may also be inferred, by comparison, that a normal body fluid level delayed this antihypertensive action. Since unclipping plus a free urine flow plus fluid replacement lowered the MAP in the same manner as UCA, indications were that the antihypertensive action of UCA was not due to the introduction of substances from the kidney via the venous anastomosis. The UCA-Na volume load-unclipping sequence is considered quite cogent with respect to ongoing considerations of the antihypertensive actions of the kidney. The only difference between the results of these experiments and those following UCA alone was in the time required for the MAP to reach normal (45-50 vs 20 hours). Thus, three time intervals were noted following unclipping, depending on the state of the body fluids: 3 hours, when body fluids were contracted; 20 hours, when body fluids were near normal; and 45-50 hours, when body fluids were expanded. Clearly, these observations represent additional indications that the antihypertensive function of the kidney modulates, in part, the influence of Na-volume on the arterial pressure. Moreover, this function is unrelated to the ability of the kidney to excrete Na and water to the outside of the body. How the state of body fluids relates to the time course involved under the different circumstances (3 to 45-50 hours) remains unknown. It is possible that greater or lesser amounts of the renal factor involved may be necessary, depending on the state of the body fluids.

UL plus a Na-volume load plus unclipping gave the same results as UL plus the same Na-volume load plus the sham procedure; in neither case did the MAP reach normal levels in 45 hours. UL, under these conditions, compromises the state of the renal papilla through hemodynamic changes. We suggested that such changes could interfere with the antihypertensive function of the papilla and its RIC.

Lucas and Floyer have proposed the existence of a factor from the kidney that modulates the state of interstitial gel compliance. According to this view, the absence of this factor, as in the renoprival state, decreases gel compliance and in the presence of a volume load elevates the arterial pressure through hemodynamic mechanisms as proposed by Guyton, et al. and Ledingham. If such a factor exists, our data on unclipping appear to support its origin from the renal papilla.

Renomedullary Deficiency and the Hypertensive State

Renoprival hypertension of the rabbit induced by the daily injection of 30 ml of saline per kg is significantly ameliorated by renomedullary transplants. While this protection transpires, body fluid compartments (blood, plasma, and ECF volumes) are expanded to the same extent as in nontransplanted hypertensive control animals (fig. 13). We interpreted this as indicating improper control of the sequelae of volume expansion, resulting from a deficiency of the antihypertensive renomedullary function.

In another setting — partial nephrectomy-salt hypertension (PN-SH) produced by the ablation of 66%-75% of renal tissue plus 1% NaCl to drink — renomedullary deficiency was demonstrated by a different approach.

After 25-30 days on the high salt intake, when the PN-SH had been well established, a morphometric study of the renal papilla demonstrated a marked reduction in RIC plus a degenerated appearance of the remaining cells. A sequential study showed that these changes were first evidenced with the first week on the high salt intake (fig. 14). Transplants of renal papillae from PN-SH rats of 14 and 25 days' duration had no effect on the MAP of one-kidney, one clip hypertensive recipients (fig. 15). The papillae from normal animals transplanted at the same time exerted the antihypertensive effect. At 7 days, the papillae of PN-SH rats displayed partial functional impairment. These functional-morphologic alterations seemed related to the high salt traffic at the sites of the RIC since PN plus a plain water intake did not disturb the RIC. It is perhaps pertinent to point out that PN-SH responds dramatically to transplants of cultured RIC.

Again, the sustained hypertensive state of this model (PN-SH) does not appear to be due solely to the Na-volume load. Rather, it seems related to a lack of adjustment to the high Na-volume intake, attendant on a deficiency of the antihypertensive function of the RIC.

It is becoming apparent that a deficiency of the antihypertensive function of the renal papilla and its RIC may transpire via different mechanisms. Two such mechanisms are provided in this section: ablation of the renal papilla through nephrectomies, and in situ damage within the kidney mediated by the salt load. In situ damage of the renal papilla by bromoethylamine with aggravation of the hypertensive state was documented by Heptinstall et al. Muehrcke et al. detected a virtual absence of RIC in malignant nephrosclerosis of man. Manthorpe found the papilla of the unclipped kidney of a two-kidney, one clip hypertensive rat to be incapable of lowering the arterial pressure of hypertensive recipients. Since the RIC are present in such a kidney, albeit having fewer lipid granules, the latter result implies some constraining effect on the function of these cells. Thus, the RIC may be deficient in its antihypertensive function through several mechanisms, including ablation, destruction, and constraint.
FIGURE 13. Left Panel: Protection is achieved against renoprival hypertension of the rabbit by autotransplants of renal medulla (open circles), as compared to nontransplanted controls (solid circles) (after 30 ml saline/kg/day s.c.). Right Panel: Changes from normal (as percentage) of various body fluid parameters show that the blood volume (Cr 51 tagged RBC), plasma volume (I-131 albumin) and ECF volume (radiosulfate) were equally expanded in both groups (140%-160%, with normal as 100%). There was no difference in serum Na concentration. The two groups had a similar depression of the hematocrit reading (60% of normal) due to a mixture of anemia and hemodilution (modified from ref. 62).

FIGURE 14. Left Panel: Elevation of the arterial pressure of animals having a sequential morphometric study of the RIC during PN-SH hypertension (mean ± SEM). Right: As the hypertension developed, the RIC decreased in number and displayed fewer lipid-containing granules (right panel) (from ref. 97).
FIGURE 15. Transplants of renal papillae from PN-SH rats into hypertensive recipients failed to lower the MAP, while similar transplants from normal rats lowered the pressure significantly (from ref. 97).

FIGURE 16. Effect of renomedullary extracts on the different hypertensive states is depicted. A. Extract prevented accelerated renoprival hypertension of the dog, while an extract of the same animal's urinary bladder did not so protect (squares). The solid circles represent the control group with renoprival hypertension (modified from ref. 98). B. Extract was given i.v. to animals with established renoprival hypertension. There were two effects: an acute depressor effect occurring immediately, and a prolonged effect followed for 48 hours (modified from ref. 98). C and D. Neutral renomedullary lipid (RM extract) was given to animals having chronic hypertension (Page and Goldblatt types); as long as it was given, the pressure remained depressed. When discontinued, the pressure gradually returned to prior hypertensive levels. (modified from ref. 104).
Antihypertensive Lipids Derived from the Renal Medulla

As soon as it became apparent that renomedullary tissue prevented "accelerated" renoprival hypertension of the dog, we investigated antihypertensive lipids, most of which, but not all, were nonprostanoid in nature.

Early Extracts

In work that began early in December, 1958, we prepared a crude extract of fresh renal medulla of the dog and tested it by the intravenous route. This renomedullary extract prevented the "accelerated" renoprival hypertension (fig. 16) while a similar extract of urinary bladder failed in this respect. The renomedullary extract also caused an acute as well as a prolonged depressor effect when injected into the hypertensive animal. In light of subsequent observations by Lee et al. and Hickler et al., the acute effect could have been due to depressor prostaglandins. We had a repetitive and simple assay but failed to capitalize on it.

Next, we coupled the injection of renin with that of the renomedullary extract in the following manner. All animals were maintained on the intravenous saline-dietary protein regimen used to evoke "accelerated" renoprival hypertension. Renin was injected intravenously each day so as to cause a pressor peak of 50 to over 100 mm Hg lasting for 5 hours or more. There were four groups: 1) normal, 2) renoprival, 3) a renoprival group receiving an extract of extrarenal tissue, and 4) a renoprival group receiving the renomedullary extract. These four groups were compared with a renoprival group subjected to the same regimen and receiving the renomedullary extract in the absence of the renin injections.

The normal group had its MAP return to baseline after each pressor peak (fig. 17). The renoprival group had the MAP return to a level that increased over the baseline each day such that the curve for renoprival hypertension was described over the 4 days under the pressor peaks. The group receiving the extrarenal extract behaved in the same manner as the renoprival group (daily pressor peaks under which the curve of "accelerated" renoprival hypertension was described). To the contrary, the renomedullary extract group behaved precisely as the group of animals with intact kidneys (normal group) — the MAP returned to baseline each day. Figure 18 summarizes these results while omitting the renin pressor peaks. Moreover, the renin-normal and the renin-renomedullary extract groups yielded results identical to those of a group

![Figure 17](http://hyper.ahajournals.org/)

**Figure 17.** Renin and renomedullary extract in "accelerated" renoprival hypertension. Each line represents results with a single example of an animal receiving the saline-protein regimen for accelerated renoprival hypertension. Left: N stands for a normal animal, RP for a renoprival animal. Right: ERE stands for a renoprival animal receiving daily i.v. an extract of extra-renal tissue, and RME stands for a renoprival animal receiving the renomedullary extract i.v. each day. All animals received an i.v. injection of renin each day so as to cause the pressor peaks shown.

Note, the pressure of the normal animal and the animal receiving the renomedullary extract returned to baseline each day. The pressure of the renoprival and renoprival-ERE animals returned to a slightly higher level each day, so as to describe the curve of accelerated renoprival hypertension under the renin pressor peaks (modified from ref. 101).
FIGURE 18. Results of the groups of experiment (shown in figure 17) but with the renin pressor peaks deleted. The renin-normal and renin-renomedullary extract yielded results identical to those of the group receiving the renomedullary extract without the renin injections (the lower three lines). The other two groups described the curves of accelerated renoprival hypertension (modified from ref. 101).

At that time (1959) we joined forces with a group at The Upjohn Company, mainly Drs. E. G. Daniels and J. W. Hinman. A series of extraction and chromatographic procedures\textsuperscript{10*10*} were applied to the crude renomedullary extract. The antihypertensive principle was demonstrated to be a lipid having chromatographic and solubility characteristics of a neutral lipid (antihypertensive neutral renomedullary lipid, ANRL\textsuperscript{10*}). Antihypertensive activity was demonstrated in dogs, rabbits, and rats having a variety of acute and chronic hypertensive states (fig. 16).\textsuperscript{103~108}

Intermediate Extracts

At that time (1959) we joined forces with a group at The Upjohn Company, mainly Drs. E. G. Daniels and J. W. Hinman. A series of extraction and chromatographic procedures\textsuperscript{10*10*} were applied to the crude renomedullary extract. The antihypertensive principle was demonstrated to be a lipid having chromatographic and solubility characteristics of a neutral lipid (antihypertensive neutral renomedullary lipid, ANRL\textsuperscript{10*}). Antihypertensive activity was demonstrated in dogs, rabbits, and rats having a variety of acute and chronic hypertensive states (fig. 16).\textsuperscript{103~108}

Recent Extracts

The neutral lipid was difficult to obtain consistently. Accordingly, we changed our extraction-purification procedures. Because the neutral lipid was suspected of being a glyceryl ether, in consultation with Drs. W. E. M. Lands and J. W. Hinman, we added a reduction step early in the process, using the reducing agent, lithium aluminum hydride — later Vitride [Na Al H\textsubscript{4} (OC\textsubscript{6}H\textsubscript{4} OCH\textsubscript{3})\textsubscript{2}]. This was followed by acetylation (theoretically to cover Positions 2 and 3). The sequence now included homogenization of fresh renal medulla followed by incubation, total lipid extraction (Bligh and Dyer\textsuperscript{109}), Vitride reduction, acetylation, and a series of chromatographic steps (column and thin layer).\textsuperscript{71}

To our amazement, by these approaches we were able to derive two classes of antihypertensive lipids — one neutral, the other polar (fig. 19).\textsuperscript{110} The neutral lipid could be derived without the Vitride step and appeared to be a natural product. The polar lipid, termed the antihypertensive polar renomedullary lipid or APRL, was obtained after Vitride and was termed semisynthetic. During the past 4 years we have concentrated our efforts on APRL. While the pursuit of the identification of ANRL continues, we have made strides in identifying APRL, both as to the class of compound and to its biologic behavior.

Antihypertensive Polar Renomedullary Lipid (APRL)

Biological Activity

Acute Depressor Effect: Given as a bolus dose intravenously to a hypertensive rat, APRL is a rapidly acting vasodilator\textsuperscript{110, 111} lowering the MAP within about 2 seconds (fig. 19). (Given in the same manner to the normal rat, the acute depressor effect is markedly attenuated. Presumably, this results from more effective arterial pressure controlling mechanisms in the normal animal, such as the baroreceptor mechanism, etc.) The magnitude and duration of this effect are dose dependent. In lower doses this effect results primarily from a decrease in peripheral vascular resistance (PVR). Given as an intravenous infusion, the MAP may be set at different levels depending on the dose.

Prolonged Depressor Effect: Given in multiple doses, APRL lowers the MAP 20 or more hours after the last input of the compound.\textsuperscript{110, 111} Depending on the dose, the MAP may require 70 or more hours to return to its pretreatment level (fig. 20). This effect also results from decreased PVR. Similar antihypertensive lipids can also be derived from RIC grown in tissue culture (fig. 21).\textsuperscript{71}

Effect on the Microcirculation: \textsuperscript{112} Superfusion of APRL (0.5 \textmu g/ml/30 secs) over the cremaster muscle of normotensive WKY rats and hypertensive SHR, 4 to 6 weeks old, revealed arteriolar dilatation. For the WKY there was a 13.6\% ± 3.5\% increase in arteriolar diameter and a 41.4\% ± 10.7\% increase in blood flow while the MAP remained constant at 83.6 ± 2.4 mm
FIGURE 19. Changes in MAP of Goldblatt hypertensive animals following the i.v. bolus injection of APRL (upper) and ANRL (lower) are demonstrated. Both give rise to an acute depressor effect but the temporal relations vary. APRL causes a sudden vasodepression while ANRL causes a more deliberate response (from ref. 110).

FIGURE 20. Prolonged depressor effect due to APRL (designated ARL) is demonstrated after three doses (total 75 μg) on two successive days. The MAP did not return to the original level until 70–90 hours after the last input of the compound(s) (from ref. 110).
Antihypertensive effect of lipids derived from the cultured RIC is also prolonged and similar to that of APRL. Solid circles represent animals receiving the lipid and the open circles represent controls receiving the vehicle. Grollman type hypertensive rats (in one-kidney, one figure 8) were used (unpublished data).

Hg (n = 15). For the SHR, the corresponding figures were 21.2% ± 4.9%, 71.4% ± 22.9%, and 117 ± 3.5 mm Hg (n = 12).

Tentative Identification of APRL: The most purified APRL available is derived by a TLC second dimension step (fig. 22). (The details of the purification and identification of APRL as a glyceryl ether are being reported elsewhere by Byers, Leach, Lands, Snyder, Desiderio, and Muirhead, in press.) This fraction is extremely polar, being soluble in water. Its behavior on TLC in several systems supports the high degree of polarity. Field desorption mass spectrometry in two laboratories yielded regularly two peaks of 86 and 104 mass, which, according to Dr. Dominic Desiderio, are consistent with choline and choline plus an oxygen. Phosphorus analysis indicated a high concentration of this element. Treatment of APRL with phospholipase C (Sigma Chemical Co.) destroyed activity. We, therefore, believe the active compound is a phosphocholine and that this structure accounts for the polarity.

Acetylation was included in an early preparation step. Removal of the acyl group in the second position by phospholipase \( \lambda \) (rattlesnake venom as source) destroyed activity. Reacetylation recovered all of the activity. Thus, the component in the second position is considered to be acetyl.

A second Vitride treatment of this material destroys its antihypertensive activity, most likely by destroying ester linkages. (The first Vitride treatment acting on the total lipid extract of the tissue homogenate brings forth APRL activity. Why this is so, remains enigmatic. One wonders if this step could release

**Figure 22.** Flow chart of the procedures used in deriving APRL and in tentatively identifying its structure.
APRL from a protected state.) The product of this treatment, on TLC, yielded two spots of interest (fig. 23). Model compounds showed that fatty alcohols traveled to spot 5 (RF = 7.5 cm), alkyl ethers traveled to spot 3 (RF < 5 cm). Acetylation of spot 5 showed by gas liquid chromatography-mass spectrometry (GLC-MS) that the major peaks here were consistent with C16:0 and C18:1 fatty alcohols. These, we believe, were derived from esters mixed with the more potent constituents of APRL. (Using radioactive labeled esters of glycerol, we demonstrated that the Vitride reduction of ester linkages was 90% complete.)

The components of spot 3 were acetylated and also were subjected to GLC-MS (fig. 23). The results were consistent with acetylated glyceryl ethers of C16:0, C16:1, and C18:1 chain length. Any other peak could be accounted for as phthalate (a plasticizer) or as completely unrelated material. We, therefore, tentatively consider APRL as a 1-alkyl ether with acetoyl in the second position and phosphocholine in the third position.

Treatment of APRL with phospholipase A₁ (porcine pancreas as source) and phospholipase A₂ (rattlesnake venom as source) in combination destroyed the antihypertensive activity. Reacetylation recovered the entire activity (fig. 24). These results are consistent with an ether linkage in Position 1 and an acetoyl in Position 2. Treatment of APRL with phospholipase A₁ alone (Rhizopus arrhizus as source) did not destroy activity (fig. 24). (Model compounds indicated that this enzyme was active in deacylating the first position.) This is consistent with an ether linkage in the first position.

Semisynthetic Alkyl Ether Analog of Phosphotidylcholine

Recently, a semisynthetic alkyl ether analog of phosphotidylcholine was prepared from choline plasmalogen obtained from beef heart. This was accomplished by reducing the alk-1-enyl moiety to an alkyl group and hydrolyzing the sn-2 acyl moiety and replacing it with an acetoyl group. The preparation so derived is 1-0-hexadecyl-2-acetoyl-sn-glycero-3-phosphocholine, one of the alkyl ethers in APRL.

It is of interest that this prepared alkyl ether analog reproduced precisely the biologic activity of APRL (refer to figs. 19 and 20). Since it is relatively pure, its effective doses for the acute and the prolonged depressor effects were much lower. For instance, 1-6 μg gave a pronounced depressor effect in the hypertensive rat. A dose response was demonstrated at 63, 126, and 189 ng (fig. 25).

Four intravenous doses of the alkyl ether analog, totaling 12-24 μg over 2 days, were followed by a depression of the MAP that was pronounced 20 hours after the last input of the compound (fig. 26). Recovery occurred 48 to 96 hours after the last input. There was no change of body weight. Importantly, when given by mouth (a total dose of 40 to 80 μg in four doses), it also reproduced the prolonged depressor effect.
Comment

Shown in figure 27 is a schematic depiction of our proposed relationship between the kidney and the hypertensive state. A mirror image relationship is proposed between prohypertensive and antihypertensive renal actions. When the arterial pressure is reasonably controlled these forces should be in balance, depicted by the arrows.

The prohypertensive actions include activation of the renin-angiotensin (or other) pressor systems and failure to prevent Na-volume loading, depicted by the upward arrow. The antihypertensive actions include relief of Na-volume loads, depicted by the downward arrow, and activation of the antipressor endocrine system of the renomedullary interstitial cells and their proposed antihypertensive hormone.

As was the case for most hormone systems during their developmental phases, the evidence for this system is incomplete — the "proof and counterproof" of Claude Bernard is not with us, yet. However, major steps are evident. These include the tissue of origin, the indications that this tissue exerts a biologic action by conveying a message systemically, almost certainly via the bloodstream, and the extraction of compounds from the tissue capable of reproducing its biologic activity.

Pursuit of this putative hormone has led us, in consultation with Blank, Snyder, Lands and Desiderio to discover a new class of potent antihypertensive agents — the alkyl ethers of glycerol. The neutral lipid, being a natural product, is perhaps a better candidate as a hormone.

Figure 24. Enzymolysis of APRL, using phospholipase A1 and A2, gave results, as depicted, consistent with an ether linkage in the first position and an acetoxy in the second. The reasons for considering Position 3 as satisfied by phosphocholine include polarity, TLC results and field desorption results (L. W. Byers et al. in press). Phospholipase A1 failed to alter the activity of APRL.

Figure 25. The alkyl ether analog of Blank and Snyder gave an acute depressor effect identical to that of APRL. This is shown on this slide when 6 μg was injected i.v. into a hypertensive animal (upper). A dose response curve is shown also (lower). (Refer to fig. 19; adapted from ref. 113).
FIGURE 26. Prolonged depressor effect as induced by the Blank-Snyder compound (alkyl ether analog) when introduced by mouth as well as by vein in one-kidney, one clip rats. (Refer to fig. 20). Return of the MAP to the original hypertensive levels occurred 48-96 hours after the last input of the compound. There was no weight lost during the antihypertensive effect. Controls, receiving either the vehicle or another lipid, had no change in pressure (from ref. 113).

PROHYPERTENSIVE ACTIONS

\[ \text{PRESSOR SYSTEM (S):} \]
\[ \text{RENIN-ANGIOTENSIN (CORTICAL)} \]
\[ \text{No VOLUME} \]

\[ \text{VEHICLE LV. 0.4-0.8 ml} \]
\[ \text{ORAL ROUTE TOTAL 20-80 µg} \]
\[ \text{IV ROUTE TOTAL 12-24 µg} \]

\[ M \pm \text{SEM} \]

FIGURE 27. A schematic depiction of our proposed relationship between the kidney and the hypertensive state (see text) (from ref. 71).
We hope that the experimental manipulations of renomedullary tissue and the therapeutic implications of the antihypertensive agents derived from this tissue, as herein discussed (fig. 28), will reinforce a conceptually balanced approach to the role of the kidney in blood pressure control.

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