Degranulation of Renomedullary Interstitial Cells During Reversal of Hypertension

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SUMMARY It was demonstrated earlier that the renal venous effluent of one-kidney, one clip hypertensive rats contained a vasodepressor lipid resembling the antihypertensive neutral renomedullary lipid (ANRL), following unclipping and as the arterial pressure (MAP) was lowered. Consequently, the sham-undipped (clip-intact) and the undipped kidney (CK and UCK) were studied by electronmicroscopy and morphometrically (Weibel's techniques). Renomedullary interstitial cells (RIC) of the CK had abundant granules. The collecting duct (CD) had tall lining cells containing pale granules and displayed intercellular channels. Following unclipping, the RIC degranulated and the CD cells became flattened, lost their pale granules, and the intercellular channels disappeared as the MAP decreased. These changes were evident by EM appearance and volume density measurements. The renopapillary changes occurred as the kidney secreted the ANRL-like substance into the blood. (Hypertension 3 (suppl II): II-75-II-80, 1981)

KEY WORDS • undipping • renal venous effluent • antihypertensive neutral renomedullary lipid (ANRL) • antihypertensive function • ureterocaval anastomosis • volume density • collecting duct • alkyl ethers

REMOVAL of the renal artery clip (unclipping) of the one-kidney, one clip hypertensive rat is followed by return of the arterial pressure to normal (baseline) levels.1 The rate of the recession of the arterial pressure appears to be dependent on the circumstances at the time of the unclipping. Unclipping when the mean arterial pressure (MAP) was at its established hypertensive level, plus a free flow of urine, was followed by the MAP reaching baseline within 3 hours.2 Diuresis-natriuresis occurred under these conditions. Unclipping plus ureterocaval (vena caval) anastomosis (UCA) was attended by a drop in MAP to baseline in an average of 20 hours.2 Under the latter conditions, there was no loss of sodium and water to the outside of the body.

It was reasoned that, should the kidney lower the MAP after unclipping by secreting an antihypertensive hormone, as our hypothesis demands,3 then at some point during the lowering of the MAP after UCA plus unclipping the renal venous effluent should contain a detectable amount of the hormone. Indeed, recently4 we demonstrated the presence of a vasodepressor lipid in the renal venous effluent as the kidney lowered the MAP following UCA plus unclipping; i.e., at an average of 5 hours after unclipping. This lipid had the chromatographic characteristics of the antihypertensive neutral renomedullary lipid (ANRL) derived from fresh renal medulla. These findings suggested that ANRL could be the sought after antihypertensive hormone of the renomedullary interstitial cells (RIC).4

If this lipid comes from the RIC, as our previous studies suggest, then a morphologic study of the renal papilla after unclipping seemed indicated. Therefore, we studied the renal papilla of the one-kidney, one clip hypertensive rat with UCA and following either unclipping or with clip left in place. The unclipped kidney was studied at two time intervals: 5 hours later when the MAP had dropped significantly but not to baseline (an average of -34 mm Hg) and 20 hours later when the MAP was at baseline.

Material and Methods

The hypertensive rats were studied about 6 months after a clip had been applied and the opposite kidney removed (MAP 191 ± 5 mm Hg). The techniques for clipping the renal artery and removing one kidney,
measuring the MAP, establishing the UCA, and unclipping have been previously described. At an average of 20 hours after either unclipping or sham operation, the remaining kidney was removed, and without further manipulation of the rat, it was subjected to morphometric study. For another group, at an average of 5 hours after unclipping, the renal venous effluent was collected for 10-20 minutes until the animal collapsed while an infusion of saline diluted with rat blood was introduced via the jugular vein to maintain blood volume. Then the kidney was harvested for the study. The sham unclipped animals were similarly treated but did not have the clip removed. The extraction procedure and chromatographic purification destined to derive ANRL from the renal venous plasma have been described elsewhere.

**Morphometric Study**

The kidneys were bisected slightly to one side of the midline so that the entire renal papilla remained attached to one half. The inner tip of the papilla was cut off for 1 ml. The next 2 ml of the papilla, approximately to where the inner medulla widens, was cut longitudinally into four slices and fixed in cacodylate buffered 3% glutaraldehyde (560 mOsM (fig. 1). The slices were then postfixed in osmic acid, dehydrated, and embedded in epoxy resin so that transverse sections could be made of each block, showing cross sections of the collecting ducts, vasa recta, and Henle's loops, as well as RIC.

Grids were examined from each of the four blocks, and a total of 30 prints was made in approximately equal numbers from each block. The area densities of the structures of the papilla, the collecting duct, Henle's loop, vasa recta, and interstitial space were evaluated using the MOP-III modular system for quantitative digital image analysis, determining the profile area of each structure as a percentage of the total papillary area. In addition, the collecting duct lumens, collecting duct cells, RIC, RIC granules, and RIC nuclei were evaluated. By the principle of Delesse these areal densities are equivalent to volume densities (Vv) of these structures. The numerical density (Nv) of RIC nuclei and RIC granules was calculated in number/mm² of papillary tissue by using the formula of Weibel and Gomez:

\[
N_v = \left(\frac{K}{\beta}\right) \frac{N_a^{1/3}}{V_v^{1/3}}.
\]

The number per unit area (Na) of these structures is what has been reported in most previous studies of RIC granulation, and volume density (Vv) was as calculated above. The factor K depends on the relative size distribution of the particles; K can be assumed to be 1.0 with no more than a 7% error if the standard deviation of particle diameter is no more than 25% of the mean diameter. The coefficient depends on shape and is 1.38 if the particles are assumed to be spherical, as they were in this study.

**Results**

Table 1 summarizes the results of this morphometric study. When the unclipped and sham-unclipped (clip intact) groups were compared, similar changes were noted whether the study was performed 5 or 20 hours after the unclipping. The most striking changes occurred in the collecting duct and the RIC granules.

There was no difference in the volume density of the interstitium, Henle's loop, or the vasa recta (not included in table). Following unclipping, there was a significant decrease in the volume density of the collecting duct due to changes in the cells rather than changes in luminal size. These changes are evident from visual inspection even without confirmation by morphometric measurements. A picture of a normal rat papillary collecting duct from an area similar to those studied morphometrically is included for comparison (fig. 2). The papillary collecting duct cells of the clipped kidney were tall, contained pale granules toward their bases, and had prominent lateral intercellular channels (fig. 3). Following unclipping, whether at five or at 20 hours, the cells became flattened, lost the pale basilar granules, and the intercellular channels disappeared (Fig. 4).

Changes also occurred in the RIC as the MAP decreased following unclipping. Both the number of RIC granules and the volume density of the granules were markedly lowered following unclipping (Figs. 3, 4, and 5). Thus, as the kidney performed its antihypertensive function, the RIC degranulated. In addition, the RIC themselves shrank slightly, at least partially related to loss of granulation but possibly including some cytoplasmic loss. The number of RIC nuclei per unit volume of tissue remained the same in the 5-hour study. However, a slight increase in RIC nuclei was noted after 20 hours. Since it seems unlikely that these
Table 1. Morphometric Data on Renal Papilla

<table>
<thead>
<tr>
<th></th>
<th>Volume density</th>
<th>No./mm³</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>IS</td>
<td>CD</td>
<td>CDL</td>
</tr>
<tr>
<td>Model 1: UCA 20 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clip intact</td>
<td>5</td>
<td>0.3497</td>
<td>0.2879</td>
<td>0.0649</td>
</tr>
<tr>
<td>p NS &lt;0.05 NS &lt;0.05 &lt;0.005 &lt;0.005 &lt;0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unclipped</td>
<td>5</td>
<td>0.3434</td>
<td>0.2261</td>
<td>0.0813</td>
</tr>
<tr>
<td>Model 2: UCA 5 hrs</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clip intact</td>
<td>5</td>
<td>0.3173</td>
<td>0.3347</td>
<td>0.0724</td>
</tr>
<tr>
<td>p NS &lt;0.025 NS &lt;0.01 &lt;0.005 &lt;0.005 NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unclipped</td>
<td>5</td>
<td>0.3163</td>
<td>0.2732</td>
<td>0.0728</td>
</tr>
</tbody>
</table>

Abbreviations: IS = interstitium, CD = collecting duct, CDL = collecting duct lumen; RICN = renomedullary interstitial cell nucleus; CDC = collecting duct cell; RIC = renomedullary interstitial cell; and RICG = renomedullary interstitial cell granules.

Discussion

The most prominent finding in the papilla of the established one kidney-one clip hypertensive rat following unclipping was degranulation of the RIC. Since antihypertensive lipids can be derived from the renal papilla and from RIC grown as monolayer tissue culture, the degranulation is consonant with the concept that these cells are the source of antihypertensive lipids. It also appears plausible that the vasodepressor lipid, resembling ANRL, derived from renal venous effluent as the MAP dropped following unclipping, was also secreted by the RIC.

The coupling of degranulation of RIC and the presence of an ANRL-like substance in renal venous effluent as the MAP was lowered supports the concept that the kidney, through its renal papilla and RIC, secretes an antihypertensive hormone. As demonstrated elsewhere, this substance is distinct from prostaglandins and the alkyl ethers of phosphatidylcholine. Thus, it appears unique. What is not known is whether the alkyl ethers of phosphatidylcholine can be transformed into ANRL. This possibility remains since the antihypertensive action of the alkyl ethers when given by mouth resembles that of transplants of RIC and that of ANRL. Additional work is needed in this area to resolve these possibilities.
FIGURE 3. The collecting ducts of the clipped kidney were lined by tall cells having light staining granules, especially between the basement membrane and the nucleus, and displayed intercellular channels EM $\times$ 3200.

FIGURE 4. Following unclipping, the cells lining the collecting duct shrank in size while the duct lumen remained the same; the light staining granules and intercellular channels disappeared; and the RIC degranulated EM $\times$ 1900.
No further degranulation of the RIC was noted between 5 and 20 hours. At the latter time the pressure had normalized. Evidently no further degranulation was necessary to maintain the pressure drop. A late study many days after unclipping would be of interest to see if the granules reaccumulated.

The nature of the RIC granules is still not clear. They may contain in part the ANRL but at this time it seems more likely that they contain substrate for prostaglandin precursor synthesis and possibly for ANRL synthesis. If this is so, the evaluation of the static RIC granulation might give information about ANRL synthesis and release best when there is an acute release of ANRL and degranulation of the RIC, as in the experiment reported here.

Very striking changes were also noted in the collecting duct. Following the unclipping procedure the columnar cells of the collecting duct became smaller and flattened out, the lateral intercellular channels noted in the clipped kidney disappeared, and basilar pale lipid granules present in the clipped kidney disappeared. The mechanism of these changes is not clear from this study. The RIC could exert an influence on the collecting duct or the collecting duct changes could be related to papillary blood flow, papillary interstitial osmolarity, intrarenal angiotensin II concentration, or any of the other host of variables which might be altered by the unclipping process.

The difference between the clipped kidney and a normal unmanipulated rat kidney was not systematically investigated in this study. A few comments seem in order, however. By visual inspection the collecting duct cells appear taller than in the unmanipulated kidney, they contain light staining lipid granules in their basilar portions, and open lateral intercellular channels were prominent. Such channels have been noted between papillary collecting duct cells in the Brattleboro rat having hereditary diabetes insipidus after treatment with ADH. Thus, the presence of the channels in the clipped kidney could relate to greater fluid absorption by the collecting duct in the clipped kidney. The loss of these channels after unclipping could indicate a decreased resorption of fluid with the greater output of urine which is observed following unclipping. This point needs further study.

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