Pressor Polypeptides Formed in Vivo and in Vitro as Mediators of Renal Hypertension

By E. Braun-Menéndez, M.D., and A. C. Paladini, Ph.D.

During the purification of hypertensin formed in vitro, evidence of heterogeneity of the pressor activity was obtained by counter current distribution. Pepsitensin shows a similar heterogeneity. These facts lead to the hypothesis that other proteolytic enzymes may also be present in the tissues and may hydrolyze hypertensinogen with the formation of pressor polypeptides of the hypertensin or pepsitensin type. Thus, substances similar to hypertensin may be formed in the organism by the action of proteolytic enzymes other than renin and may, if produced locally, exert a vasoconstrictor action or, if they enter the circulation, cause an increase in blood pressure.

The importance of chemical mediators in normal and pathologic conditions is now universally recognized. The term "local hormones" was introduced not long ago by Professor Gaddum and is applied to "pharmacologically active substances [having] important local functions in the regulation of tissue activity, particularly of involuntary muscles, gland cells and capillaries." This term does not include those substances which are set free in normal or injured tissue and may act on distant organs. Many of these occur naturally and may be considered as true hormones, while others have been proved to be formed only under artificial conditions and their function in physiologic or pathologic conditions is thus only hypothetical.

The polypeptides form an important group of these substances. Recently pharmacologically active organic acids have been shown to be formed in vitro and in vivo and some of them have vasoconstrictor and pressor activities. Table 1 gives a list of some pharmacologically active polypeptides. All of those formed in vitro by the action of proteolytic enzymes have the same substrate (α-globulin). Only vasopressin, hypertensin, and pepsitensin have a pressor action when injected intravenously.

Vasopressin has been purified, its composition and amino acid sequence recognized, and its synthesis achieved. We know, nevertheless, that there are at least 2 vasopressins according to their origin (hog or bovine) and differing only by the presence or absence of 1 amino acid.

Hypertensin, or more correctly, some of the hypertensins, have been purified and 2 of them synthesized. During the purification of hypertensin prepared with hog renin and ox hypertensinogen, evidence of heterogeneity of the pressor activity was obtained by countercurrent distribution in the systems 2-butanol, 0.1 M ammonium hydroxide and 2-butanol, 0.05 M sodium phosphate buffer pH 7.65. These results led to the study of the countercurrent behavior of hypertensin preparations obtained with hypertensinogen from different animal species and hog renin. The results (fig. 1) show that hog, horse, and ox hypertensinogens each give rise to several active components on incubation with hog renin. A similar heterogeneity was found in the hypertensin formed in the plasma of a dog after total renal ischemia of 6 hours' duration. The plasma was incubated at 2 C. for 24 hours. With some reservations it may be tentatively concluded that circulating hypertensin is also heterogeneous.

At present it is difficult to explain the origin and physiologic importance of the hypertensins reported here, but it seems fairly well established that the preparations currently employed as starting materials for the purification may consist of complex mixtures. Attempts to distinguish some of the separate components pharmacologically have been unsuccessful up to now. Neither the pretreatment of the Nembutalized rat by different
PRESSOR POLYPEPTIDES AND RENAL HYPERTENSION

Table 1.—Pharmacologically Active Polypeptides

<table>
<thead>
<tr>
<th>Active polypeptides</th>
<th>Chymotrypsin</th>
<th>Trypsin</th>
<th>Pepsin</th>
<th>Action on B.P.</th>
<th>Other actions</th>
<th>Enzymes</th>
<th>Substrate destroyed by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasopressin</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>anti-diuretic</td>
<td>pepsin</td>
<td>alpha-globulin</td>
</tr>
<tr>
<td>Pepsanurin</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>anti-diuretic</td>
<td>pepsin</td>
<td>alpha-globulin</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>uterus+</td>
<td>pepsin</td>
<td>alpha-globulin</td>
</tr>
<tr>
<td>Pepsitocin</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>uterus+</td>
<td>pepsin</td>
<td>alpha-globulin</td>
</tr>
<tr>
<td>Substance P</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>gut+</td>
<td>pepsin</td>
<td>alpha-globulin</td>
</tr>
<tr>
<td>Substance U</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>-</td>
<td>gut &amp; uterus+</td>
<td>alpha-globulin</td>
<td>?</td>
</tr>
<tr>
<td>Kallidin</td>
<td>+</td>
<td>0</td>
<td>?</td>
<td>-</td>
<td>gut &amp; uterus+</td>
<td>kallikrein</td>
<td>alpha-globulin</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>+</td>
<td>0</td>
<td>?</td>
<td>--</td>
<td>gut &amp; uterus+</td>
<td>trypsin</td>
<td>alpha-globulin</td>
</tr>
<tr>
<td>Hypertensin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>gut &amp; uterus+</td>
<td>renin</td>
<td>alpha-globulin</td>
</tr>
<tr>
<td>Pepsitensin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>gut &amp; uterus+</td>
<td>pepsin</td>
<td>alpha-globulin</td>
</tr>
</tbody>
</table>

Substrate destroyed by:
- Previous renin
- Heat
- Alcohol ppt.
- pH 3.9

<table>
<thead>
<tr>
<th>Active polypeptides</th>
<th>Inactivated by</th>
<th>Trypsin</th>
<th>Pepsin</th>
<th>Action on B.P.</th>
<th>Substrate destroyed by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasopressin</td>
<td>Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Pepsanurin</td>
<td>Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>Trypsin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>uterus+</td>
</tr>
<tr>
<td>Pepsitocin</td>
<td>Trypsin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>uterus+</td>
</tr>
<tr>
<td>Substance P</td>
<td>Trypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>gut+</td>
</tr>
<tr>
<td>Substance U</td>
<td>Trypsin</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>gut &amp; uterus+</td>
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<tr>
<td>Kallidin</td>
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<td>+</td>
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<td>+</td>
<td>0</td>
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</tr>
<tr>
<td>Hypertensin</td>
<td>Trypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>gut &amp; uterus+</td>
</tr>
<tr>
<td>Pepsitensin</td>
<td>Trypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>gut &amp; uterus+</td>
</tr>
</tbody>
</table>

Fig. 1. Counter current distribution of different preparations of hypertensin. **Solid line**, experimental curve; **dotted lines**, theoretical distribution of a single substance; **Hog-Hog**, hog renin and hog hypertensinogen; **Hog-Horse**, hog renin and horse hypertensinogen; and so on. The pressor activity was measured in nephrectomized rats anesthetized with Nembutal. One rat unit is the pressor activity of 0.2 ml. of a standard hypertensin preparation which raises the blood pressure 20 to 35 mm. Hg and is equivalent approximately to 0.01 Goldblatt units. (Reprinted from Biochem. et Biophys. Acta 18: 580, 1955.)

drugs, nor parallel assays of vasoconstrictor and plain muscle stimulating action, in order to find a significant "index of discrimination," have given definite results.13

Pepsitensin is the product of hydrolysis of hypertensinogen (α-2-globulin) by pepsin.

If the mixture of hypertensinogen and an adequate amount of pepsin is incubated a certain time, acidified to pH 5 with dilute HCl, and then brought to boiling in a water bath, a maximum production of pepsitensin is obtained independently of the time and temperature of incubation. Concentration of the enzyme and pH of the mixture, on the other hand are variables which determine the amount of pepsitensin formed. It was found that 3 to 5 mg. of crystallized pepsin per ml.
of hypertensinogen is the optimum concentration (1 ml. of hypertensinogen is equivalent to 3 ml. of original plasma). At this concentration, maximal production of pepsitensin changes with pH of the mixture, showing maxima at approximately pH 4 and at pH 6.3. As was known, the proteolytic activity measured by a modified Anson's method diminished with increasing pH (fig. 2).

A given amount of hypertensinogen yields equivalent pressor units of hypertensin or pepsitensin when treated with renin or pepsin under optimal conditions (table 2). Pepsin is able to form nearly maximal amounts of pepsitensin (80 per cent) when acting upon heat-coagulated hypertensinogen, and about 15 per cent of the normal production with heat-coagulated hypertensinogen previously treated with renin. Evidence of heterogeneity of the pressor activity of pepsitensin preparations was also obtained by counter current distribution (fig. 3).

The fact that a maximum of pepsitensin formation was observed at pH 6.3 led to the hypothesis that other proteolytic enzymes present in the tissues might hydrolyze hyper-

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**TABLE 2.—Maximal Production of Hypertensin and Pepsitensin**

<table>
<thead>
<tr>
<th>Renin mg./ml. Hgen* pH 7.5</th>
<th>Rat U. per ml. Hgen</th>
<th>Pepsin mg./ml. Hgen pH 4.3</th>
<th>Rat U. per ml. Hgen</th>
<th>Pepsin mg./ml. Hgen pH 6.3</th>
<th>Rat U. per ml. Hgen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>104</td>
<td>3.0</td>
<td>100</td>
<td>3.0</td>
<td>100</td>
</tr>
<tr>
<td>0.3</td>
<td>92</td>
<td>3.0</td>
<td>94</td>
<td>4.5</td>
<td>93</td>
</tr>
<tr>
<td>0.5</td>
<td>104</td>
<td>—</td>
<td>—</td>
<td>6.0</td>
<td>120</td>
</tr>
<tr>
<td>1.0</td>
<td>112</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Av. 103</td>
<td>97</td>
<td>104</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Bovine hypertensinogen: 1 ml. equivalent to 3 ml. of original plasma. Time of incubation at 30 C. for renin, 20 min. and for pepsin 5 or more min. Results are expressed in "rat units" of pressor activity, approximately equivalent to .01 dog (Goldblatt) units. Renin solution used contained 1 dog unit per ml.
tensinogen with the formation of pressor polypeptides of the hypertensin or pepti
dsin type. Extracts of spleen were prepared according to the method of Fruton and Berg-
mann\textsuperscript{14} for cathepsin, and incubated with hypertensinogen. Experiments are still in progress, but in spite of some encouraging preliminary results, nothing definite can be stated regarding the production of pressor polypeptides by this reaction.

Recently Dengler\textsuperscript{15} has presented evidence that the arterial wall contains a thermolabile, nondialyzable factor precipitated with ammonium sulphate between 0.3 and 0.6 saturation which, when incubated with plasma globulin, yields a substance with vasoconstrictor and pressor activity and which contracts the rat’s uterus and the guinea pig’s ileum. It could be argued that the arterial wall could contain renin, as suggested by Introzzi et al.,\textsuperscript{16} but apparently the arterial wall extract is not identical with renin. No definite proof is yet available of the polypeptide nature of the product of its reaction with plasma globulins; nevertheless this possibility must be borne in mind.

It does not seem illogical to assume that the production of pharmacologically active sub-
stances by the action of tissue enzymes on blood substrates may be one of the homeo-
static mechanisms of the body. The formation of polypeptides by the action of proteolytic
enzymes on blood globulin (especially \(\alpha_2\)-globulin) is one of such mechanisms. Many polypeptides have been studied and there may be many more. The physiologic significance of bradykinin or bradykinin-like substances has recently been recognized as playing a role in changes of capillary permeability, production of local pain, and vasoconstriction.\textsuperscript{17} The facts discussed in this paper suggest that sub-
stances similar to hypertensin may be formed by the action of proteolytic enzymes other than renin and may, if produced locally, exert a vasoconstrictor action or, if they enter the circulation, produce an increase in blood pressure.

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