ENDOTHELIN AND SARAFOTOXIN PRODUCE DISSIMILAR EFFECTS ON RENAL BLOOD FLOW, BUT BOTH BLOCK THE ANTIDIURETIC EFFECTS OF VASOPRESSIN[1]

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Abstract. Human endothelin, a 21-residue peptide produced by vascular endothelial cells, was infused intravenously into trained conscious dogs at a rate of 20 $ng \cdot kg^{-1} \cdot min^{-1}$ for 1 hr. Endothelin produced a renal vasoconstriction that persisted during a 40-minute recovery period. Sarafotoxin S6b, a closely related 21-residue peptide that has been isolated from the venom of the burrowing asp, was also infused into the same conscious dogs at 20 $ng \cdot kg^{-1} \cdot min^{-1}$. Sarafotoxin produced a renal vasodilation that persisted throughout the infusion; when the infusion ended, however, renal blood flow decreased rapidly to below control levels. Both endothelin and sarafotoxin produced marked decreases in urine osmolality even though plasma vasopressin remained normal, thus indicating that these peptides inhibit the antidiuretic effects of vasopressin. These results imply that a broad spectrum of structure-activity relationships may exist among analogues of this unique group of 21-residue peptides.

Endothelin is a recently-discovered peptide that is produced by vascular endothelial cells and is capable of eliciting marked effects on vascular tone (1). Close structural and functional similarities between endothelin and a group of peptide toxins (sarafotoxins) isolated from the venom of the burrowing asp, Atractaspis egaddensis, recently were noted (2,3). Both endothelin and the sarafotoxins are 21-residue peptides having two disulfide bonds. There is a high degree of homology between the endothelin and sarafotoxin peptides; fourteen of the amino acids in the sequence of human/porcine endothelin are identical to those in sarafotoxin S6b.

We recently described several cardiovascular, renal, and endocrine effects that are elicited by the infusion of small amounts of endothelin into conscious dogs (4). Renal blood flow was not measured in that study. We now report preliminary results from a study in which we compared the effects of intravenously administered sarafotoxin and endothelin on renal blood flow and urine osmolality in conscious dogs.

Materials and Methods. Renal blood flow was measured in four conscious dogs with a transit-time flow probe (Transonic Systems, Ithaca, NY) that had been positioned around the left renal artery; cardiac output was measured with a transit-time flow probe placed around the ascending aorta. The probes were implanted during a surgical operation performed under general anesthesia (pentobarbital sodium, 25 mg/kg i.v., with supplements given as needed) at least two weeks before the experiments were conducted. Arterial blood pressure was measured by means of an indwelling catheter connected to a transducer. Renal blood flow, arterial blood pressure, and cardiac output signals were digitized by computer at a sampling rate of 100 Hz. After a 40-min control period, human porcine endothelin (Peninsula Laboratories, Belmont, CA, lot #017127) and, on a separate day, sarafotoxin S6b (Peninsula Laboratories, lot #017338) were dissolved in isotonic saline and infused intravenously into the conscious dog at a rate of 20 $ng \cdot kg^{-1} \cdot min^{-1}$ for 1 hr. Each infusion was followed by a 40-min recovery period. An interval of at least three days was allowed between experiments, and the peptide to be infused in each experiment was selected by randomization. Urine was collected at 20 min intervals via a self-retaining bladder catheter. Blood samples for measurement of plasma vasopressin were obtained 5 min before the end of the control period, after 30 and 55 min of infusion of either peptide, and 5 min before the end of the recovery period. Plasma vasopressin was measured by radioimmunoassay as described previously (5). Urine osmolality was measured by freezing point depression (model 3MO, Advanced Instruments, Needham Heights, MA).

Results and Discussion. Fig. 1 illustrates the typical decreases in urine osmolality that occurred during 1 hr infusions of sarafotoxin S6b and, on another day, endothelin into the same conscious dog. Urine osmolality decreased to 162 mosm/kg H_2O during the sarafotoxin S6b infusion and to isotonicity during the infusion of human/porcine endothelin. These changes occurred even though plasma vasopressin levels did not decline from their respective control levels of 4.7 and 5.0 pg/ml; these concentrations of vasopressin are markedly antidiuretic in the conscious dog as illustrated by the high values of urine osmolality in the control period. Urine flow increased 10-fold during the sarafotoxin infusion and 3-fold during the endothelin infusion. Combined data from experiments on four dogs revealed that sarafotoxin S6b decreased urine osmolality from a control value of 1199 $\pm$ 122 (mean $\pm$ SE) to 236 $\pm$ 67 mosm/kg H_2O ($p<0.01$), and endothelin decreased urine osmolality from 1208 $\pm$ 84 to 279 $\pm$ 37 mosm/kg H_2O ($p<0.01$). Urine flow increased from 0.42 $\pm$ 0.08 to 3.48 $\pm$ 1.02 ml/min ($p<0.01$)

[1]This work was supported by USPHS grants HL13623 and HL38139.

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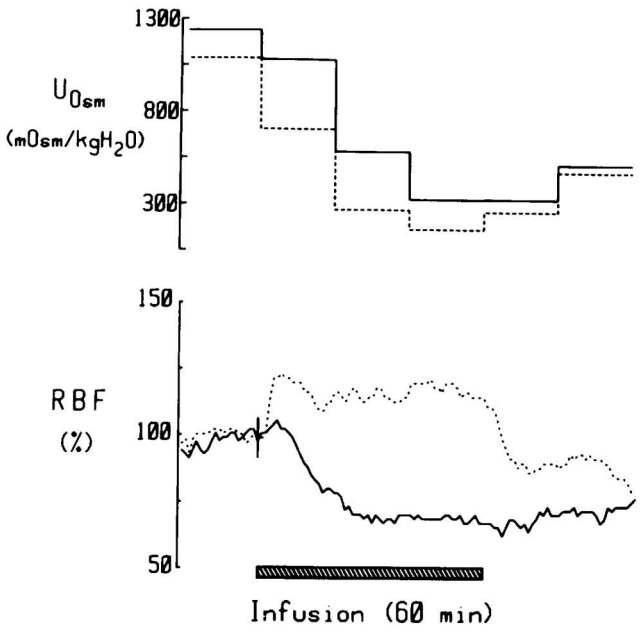

Fig. 1. Representative experiment illustrating changes in urine osmolality (UOsm) and renal blood flow (RBF) during infusion of endothelin (solid lines) and sarafotoxin S6b (dotted lines) into the same trained conscious dog.

in response to sarafotoxin and from 0.42 ± 0.12 to 1.16 ± 0.30 ml/min ($p<0.01$) in response to endothelin. Vasopressin levels were unchanged from their control levels of 3.1 ± 0.8 and 2.6 ± 0.9 pg/ml for sarafotoxin and endothelin, respectively.

We conclude that sarafotoxin S6b and endothelin are highly effective antagonists of the antidiuretic effects of vasopressin. Although this antagonism conceivably could be mediated by a direct effect of these peptides on vasopressin V_2 receptors in the renal collecting ducts, another possibility appears to be more likely. Endothelin and sarafotoxin are capable of initiating the hydrolysis of phosphatidylinositides in some organs (6,7), and the breakdown of phosphoinositides is known to modulate the classic cyclic AMP-mediated hydro-osmotic action of vasopressin through a number of pathways (8). Specifically, receptor-mediated activation of phospholipase C hydrolyzes phosphatidylinositol biphosphate; two products of this hydrolysis, inositol triphosphate and diacylglycerol, are responsible for the resultant increase in intracellular calcium and activation of protein kinase C, respectively. Each of these latter events inhibits the effects of vasopressin on water reabsorption in the collecting duct cell and also leads to an increased concentration of arachidonic acid which can inhibit vasopressin-mediated water transport via the prostaglandins (see reference 8 for a summary of current concepts). To our knowledge, an effect of endothelin and sarafotoxin on the hydrolysis of phosphatidylinositide in renal collecting duct cells has not been reported, but such an effect has been noted in rat atria and brain (6,7). Moreover, binding sites for endothelin are quite numerous in the medulla and papilla of the rat kidney (9). We therefore speculate that endothelin and sarafotoxin bind to receptors in renal collecting ducts and thereby activate phosphlipase C to initiate the breakdown of phosphatidylinositol biphosphate and that this process was responsible for blocking the hydro-osmotic effects of vasopressin in the present experiments. If this interpretation proves to be correct, our data would indicate that this process, which has been studied primarily *in vitro* (8), can exert dramatic effects on water excretion in normal conscious animals.

Sarafotoxin S6b consistently caused an increase in renal blood flow that began within 3-4 min and gradually returned toward control values during the infusion period (see Fig. 1). Renal blood flow decreased to below control levels immediately after the infusion ended. In contrast, endothelin caused a progressive decline in renal blood flow during the infusion period, and the flow remained low throughout the 40-min recovery period (see Fig. 1). Blood flow to the left kidney increased from a control level of 248 ± 26 to 299 ± 35 ml/min ($p<0.05$) during infusion of sarafotoxin, and decreased from 240 ± 11 to 186 ± 19 ml/min ($p<0.01$) during infusion of endothelin.

Arterial blood pressure increased significantly in response to the endothelin infusion (from 108 ± 1 mmHg in the control period to 129 ± 6 mmHg during the last 20 min of the 1 hr infusion period, $p<0.01$) and remained significantly elevated throughout the recovery period. Blood pressure was unchanged during the sarafotoxin infusion. Total peripheral resistance was increased by 54% ($p<0.01$) during the infusion of endothelin and by 16% (NS) during the infusion of sarafotoxin. During the last 20 minutes of the recovery period, however, the mean elevation of total peripheral resistance over the control value in the sarafotoxin experiment (+42%, $p<0.05$) was virtually identical to the mean elevation in the endothelin experiment (+39%, $p<0.05$).

Endothelin is known to activate processes that cause opposing effects on blood vessels (10): a vasoconstriction that is long lasting and may be mediated by activation of dihydropyridine-sensitive calcium channels (1) or by activation of phospholipase C and the subsequent hydrolysis of phosphatidylinositol biphosphate (11) to activate the pathways mentioned above. However, endothelin also can produce a vasodilation that may be mediated by the release of short-acting vasodilators such as prostacyclin and endothelium-derived relaxing factor (10). Our data are consistent with the notion that sarafotoxin S6b also may be capable of producing either vasodilation or vasoconstriction. In the renal vasculature of the conscious dog, endothelin (in the dose employed in this study) appears to produce predominantly vasoconstriction and sarafotoxin predominantly vasodilation. It seems quite likely, however, that the decrease in renal blood flow to below control levels after the sarafotoxin infusion had ended reflected persistent vasoconstrictor effects of this peptide that became evident as effects of short-acting vasodilators dissipated. The increase in total peripheral resistance that occurred in the recovery period after the infusion of sarafotoxin had been stopped is consistent with this possibility.

The differences in biological responses elicited by porcine/human endothelin and

sarafotoxin S6b reported here imply that a broad spectrum of structure-activity relationships may exist among analogues of this unique group of 21-residue peptides. Further characterization of the biological effects of these peptides may provide greater insight into the regulatory mechanisms that influence vascular tone and the renal regulation of body fluids.

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Received May 8, 1989. [P.S.E.B.M. 1989, Vol 191]
Accepted May 23, 1989.

0037-9727/89/1914-0425$2.00/0