Renal Actions of Endothelin during Mannitol and Saline Expansion (43445)

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Abstract. **We evaluated the effects of volume expansion with saline (0.5 ml kg^{-1} min^{-1}, n = 13) and with 10% mannitol in saline (0.5 ml kg^{-1} min^{-1}, n = 13) on the cardiorenal actions of endothelin-1 (ET) in rats anesthetized with sodium pentobarbital. We also evaluated to what extent the calcium channel antagonist, verapamil (0.02 mg kg^{-1} min^{-1}), altered the cardiorenal actions of endothelin in volume-expanded rats (n = 10 with saline and n = 10 with mannitol). In five rats from each group, renal blood flow was measured with an electromagnetic flow probe. Sixty minutes after surgery, control clearances were collected, ET (110 ng kg^{-1} min^{-1}) was then infused for 30 min, and recovery clearances were collected for 60 min. ET caused a similar increase in mean arterial blood pressure and decrease in renal blood flow and the glomerular filtration rate in the saline and mannitol groups. Verapamil significantly attenuated but did not abolish the ET-induced increase in mean arterial blood pressure in both saline- and mannitol-treated rats. By contrast, the calcium channel antagonist had no effect on the ET-induced decrease in either the glomerular filtration rate or renal blood flow in saline-treated rats, but significantly attenuated these responses to ET in mannitol-expanded animals. These data demonstrate that (i) the systemic and renal responses to ET are not affected by expansion with saline or mannitol and (ii) the renal vasoconstriction prompted by endothelin is not affected by verapamil in saline-expanded rats, but is attenuated by the Ca^{2+} channel antagonist during expansion with mannitol. These data suggest that during volume expansion with mannitol, but not with saline, the ET-induced renal vasoconstriction occurs primarily at intrarenal resistance sites that are dependent upon extracellular Ca^{2+}.** [P.S.E.B.M. 1992, Vol 200]

Endothelins are a class of peptides produced by vascular endothelial cells (1, 2) and are among the most potent endogenous vasoconstrictors currently isolated. Intravenous infusions of endothelin-1 cause a number of cardiovascular, renal, and endocrine responses, including increases in mean arterial pressure, plasma renin activity, plasma norepinephrine and epinephrine concentrations, and decreases in renal blood flow and glomerular filtration rate (1–4). The kidney is very sensitive to endothelin, as illustrated by the long-lasting renal vasoconstriction that occurs during infusion of the peptide (3, 5–8). The change in intrarenal resistance is the result of a constriction of both afferent and efferent arterioles (9, 10).

The kidneys may also play a major role in the metabolism and/or clearance of endothelin. Cao and Banks (11) and Kohno *et al.* (12) reported that the systemic response to endothelin, as defined by the increase in mean arterial blood pressure, is both potentiated and prolonged in bilaterally nephrectomized rats.

Mannitol, a nonelectrolyte osmotic diuretic, also has pronounced effects on the kidney. The sugar has been used to prevent, as well as to treat, acute renal failure and to reduce intracranial and intraoccular pressure (13, 14). Mannitol-induced diuresis is caused by an alteration of the osmotic gradient across the renal tubule, which results in a decrease in water reabsorption and an increase in urine flow rate. In addition, hyperosmotic solutions of mannitol have been shown to attenuate the vasoconstrictive effects of norepinephrine, prostaglandin $F_{2\alpha}$, and serotonin in isolated vascular smooth muscle strips (15, 16). These actions may account for the reported vasodilation of vascular beds caused by the sugar (16).

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Potential interactions between mannitol and endothelin have not been evaluated. Therefore, because mannitol affects both renal hemodynamics and tubular function, the present studies were designed to evaluate to what extent the osmotic diuretic affects the cardiorenal actions of endothelin. Rats expanded with saline served as controls for the mannitol-expanded animals. In addition, verapamil was utilized to examine the role of L-type Ca^{2+} channels in the endothelin-induced systemic and renal vasoconstriction during saline and mannitol expansion.

Materials and Methods

Forty-six female Sprague-Dawley rats (200–300 g) were maintained on standard rat chow and water *ad libitum* until the experiments. Rats were anesthetized with sodium pentobarbital (60 mg/kg) and rectal temperature was maintained at 37 ± 0.5°C with a radiant heat lamp connected to a temperature controller. The left femoral vein and artery were cannulated with PE-50 tubing. Blood pressure was monitored with a pressure transducer and displayed on a chart recorder. Immediately after catheterization of the femoral vein, saline containing 3% creatinine was infused at a rate of 24 μl/min and maintained throughout the experiment. A second infusion pump was utilized to infuse: saline at 0.5 ml kg^{-1} min^{-1} (Group 1, n = 13), saline + verapamil at 0.02 mg kg^{-1} min^{-1} (Group 2, n = 10), 10% mannitol in saline at 0.5 ml kg^{-1} min^{-1} (Group 3, n = 13), or mannitol + verapamil at 0.02 mg kg^{-1} min^{-1} (Group 4, n = 10) via the left femoral vein throughout the entire experiment. The bladder was cannulated with PE-100 tubing via an abdominal incision. Renal blood flow (RBF) was measured with an electromagnetic flow probe positioned on the left renal artery, as described previously (17), in five rats from each group.

After surgery, the rats were administered 1.3 mg of sodium pentobarbital, a procedure that was repeated at 20 min during a 60-min stabilization period. Three × 15-min clearance periods (C_1, C_2, and C_3) were then performed with a 0.2-ml arterial blood sample obtained after C_1; baseline values represent the average of C_1–C_3. Endothelin-1 was then added to the creatinine infusion to yield a dose of 110 ng kg^{-1} min^{-1}; this dose is one that we utilized previously (3) and represents a dose of the peptide that is approximately 2-fold greater than the minimum dose required to produce significant changes in arterial blood pressure and renal function. Endothelin was infused for 30 min during which time 2 × 15-min clearances (E_1 and E_2) were collected; the second arterial blood sample (0.2 ml) was obtained immediately following E_2. The infusion of endothelin was stopped and 3 × 20-min recovery clearances (R_1, R_2, and R_3) were collected with 0.5 ml of arterial blood obtained following R_3. Upon the completion of each experiment, all rats were sacrificed with a lethal dose of sodium pentobarbital.

Chemical Determinations

Urine volume was determined gravimetrically. Creatinine concentrations in urine and blood were measured by the method of Folin and Wu (18); the clearance of creatinine was equated with the glomerular filtration rate (GFR) (19).

Endothelin-1 (Peninsula Laboratories) was dissolved in 0.5 N acetic acid, divided into aliquots, and stored at −80°C until the experiment.

Statistical Analyses

Differences within each group were determined by using one-way analysis of variance for repeated measures and Duncan's new multiple-range test. Differences between groups were evaluated using Student's t test for grouped data. Values were accepted as significantly different when the probability of no difference was less than 5%. Means ± SE are reported.

Results

Systemic Response: Increase in Mean Arterial Blood Pressure. Endothelin-induced changes in mean arterial pressure (MAP) are summarized in Table I. In addition, since the verapamil-treated rats had significantly lower baseline MAP values compared with corresponding control groups, the MAP data were also analyzed as a percentage of the baseline (time = 0) values; these data are illustrated in Figure 1. Endothelin caused a significant increase in MAP in both saline-treated (Group 1) and mannitol-treated (Group 3) rats; the fractional increase in MAP was similar in both groups.

Verapamil significantly attenuated, but did not totally block, the endothelin-induced increase in MAP in both saline-expanded (Group 2) and mannitol-expanded rats (Group 4). Thus, as is illustrated in Table I, endothelin did induce a significant increase in MAP above the baseline value in both groups of verapamil-treated animals.

Renal Response: Decrease in GFR and RBF. The endothelin-induced changes in glomerular filtration rate, renal blood flow, and urine flow rate are also summarized in Table I. GFR and RBF values were normalized to the corresponding baseline values and these data are illustrated in Figure 2 (GFR data) and in Figure 3 (RBF data). Endothelin caused a significant decrease in both GFR and RBF in each of the groups studied. However, as is illustrated in Figure 2, the endothelin-induced decrease in GFR in saline-expanded rats was not affected by verapamil (only the GFR values obtained during the first recovery period following infusion of endothelin were significantly different from baseline). By contrast, the endothelin-in-

Table I. Endothelin-Induced Changes in MAP, GFR, RBF, and Urine Flow Rate in Each Group of Rats[a]

	Baseline	ET infusion			Recovery	
		15 min	30 min	50 min	70 min	90 min
MAP (mm Hg)						
ET + saline (n = 13)	100 ± 5	124 ± 4[b]	135 ± 4[b]	131 ± 5[b]	121 ± 6[b]	120 ± 7[b]
ET + saline + VERAP (n = 10)	79 ± 3[c]	88 ± 4[b,c]	92 ± 4[b,c]	89 ± 3[b,c]	88 ± 3[b,c]	86 ± 3[b,c]
ET + mannitol (n = 13)	105 ± 4	131 ± 3[b]	144 ± 3[b]	136 ± 4[b]	128 ± 3[b]	121 ± 2[b]
ET + mannitol + VERAP (n = 10)	88 ± 4[c]	93 ± 4[c]	101 ± 5[b,c]	98 ± 3[b,c]	95 ± 3[b,c]	92 ± 4[c]
GFR (ml/min)						
ET + saline (n = 13)	2.61 ± 0.19	1.48 ± 0.20[b]	0.64 ± 0.18[b]	1.07 ± 0.18[b]	1.45 ± 0.17[b]	1.88 ± 0.22[b]
ET + saline + VERAP (n = 10)	2.98 ± 0.28	1.81 ± 0.17[b]	0.85 ± 0.16[b]	2.44 ± 0.38[c]	2.13 ± 0.18[c,d]	1.89 ± 0.25[b]
ET + mannitol (n = 13)	2.37 ± 0.29	1.43 ± 0.16[b]	0.61 ± 0.09[b]	0.95 ± 0.08[b]	1.50 ± 0.14[b]	1.65 ± 0.20[b]
ET + mannitol + VERAP (n = 10)	2.05 ± 0.21	1.87 ± 0.21	1.33 ± 0.16[b,c]	1.21 ± 0.09[b,e]	1.80 ± 0.13	1.88 ± 0.15
RBF (ml/min)						
ET + saline (n = 5)	4.2 ± 0.6	1.9 ± 0.3[b]	1.4 ± 0.2[b]	1.9 ± 0.3[b]	2.3 ± 0.4[b]	2.5 ± 0.2[b]
ET + saline + VERAP (n = 5)	5.7 ± 0.5	3.3 ± 0.5[b,c]	2.1 ± 0.5[b]	2.9 ± 0.7[b]	3.6 ± 0.7	4.2 ± 0.6[b,e]
ET + mannitol (n = 5)	5.5 ± 0.6	1.6 ± 0.2[b]	0.7 ± 0.3[b]	1.1 ± 0.3[b]	2.9 ± 0.9[b]	2.6 ± 0.4[b]
ET + mannitol + VERAP (n = 5)	5.1 ± 0.4	3.4 ± 0.5[b,e]	2.1 ± 0.3[b,c]	2.4 ± 0.4[b]	3.3 ± 0.3[b]	3.6 ± 0.4[b]
Urine flow rate (μl/min)						
ET + saline (n = 13)	79 ± 12	94 ± 12	37 ± 11[b]	31 ± 6[b]	47 ± 8[b]	69 ± 11
ET + saline + VERAP (n = 10)	78 ± 14	89 ± 12	32 ± 8[b]	54 ± 10[d]	71 ± 11	85 ± 8
ET + mannitol (n = 13)	200 ± 17	185 ± 13	68 ± 8[b]	98 ± 8[b]	166 ± 12[b]	198 ± 17
ET + mannitol + VERAP (n = 10)	188 ± 18	170 ± 17	126 ± 20[b,c]	141 ± 18[b,e]	184 ± 16	182 ± 11

[a] Values are mean ± SE. ET, endothelin.
[b] $P < 0.01$ compared with corresponding baseline value.
[c] $P < 0.01$ compared with corresponding group without verapamil (VERAP).
[d] $P < 0.05$ compared with corresponding baseline value.
[e] $P < 0.05$ compared with corresponding group without verapamil (VERAP).

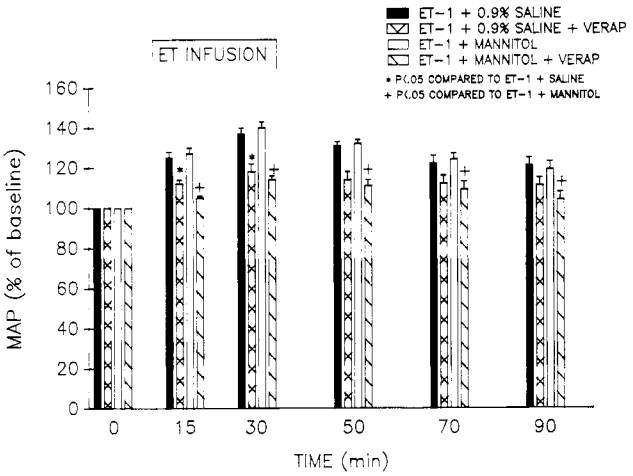

Figure 1. Mean arterial blood pressure (MAP, percentage of baseline) values are shown before, during, and after intravenous infusions of endothelin (110 ng kg^{-1} min^{-1}) into saline-expanded rats (0.5 ml kg^{-1} min^{-1}) (Group 1, solid bars), into rats treated saline + verapamil (0.02 mg kg^{-1} min^{-1}) (Group 2, cross-hatched bars), into rats treated with 10% mannitol in saline (0.5 ml kg^{-1} min^{-1}) (Group 3, open bars), and into rats treated with 10% mannitol in saline + verapamil (Group 4, right-slanted bars). *$P < 0.05$ compared with saline alone; +, $P < 0.05$ compared with 10% mannitol alone. Statistical evaluation of time-related changes in MAP in these rats are reported in Table I.

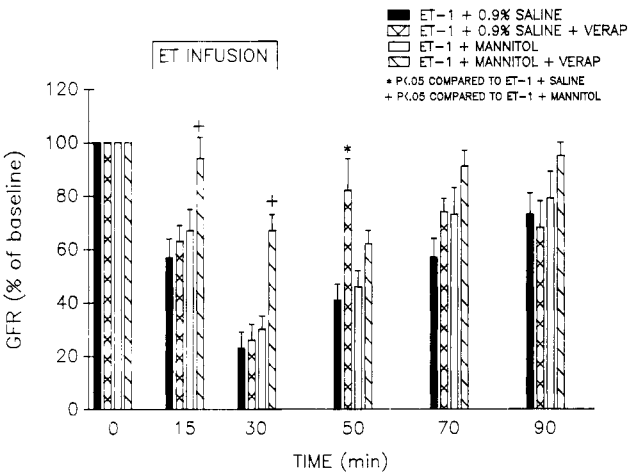

Figure 2. GFR values (percentage of baseline) are shown before, during, and after intravenous infusions of endothelin (110 ng kg^{-1} min^{-1}) into saline (0.5 ml kg^{-1} min^{-1})-expanded rats (Group 1, solid bars), into rats treated with saline + verapamil (0.02 mg kg^{-1} min^{-1}) (Group 2, cross-hatched bars), into rats treated with 10% mannitol in saline (0.5 ml kg^{-1} min^{-1}) (Group 3, open bars), and into rats treated with 10% mannitol in saline + verapamil (Group 4, right-slanted bars). *$P < 0.05$ compared with saline alone; +, $P < 0.05$ and compared with 10% mannitol alone. Statistical evaluation of time-related changes in GFR are reported in Table I.

duced decrease in GFR in mannitol-expanded rats was significantly attenuated by verapamil. Similarly, verapamil had no effect on the endothelin-induced decrease in RBF in saline-expanded rats, but significantly attenuated the renal hemodynamic response to the peptide in mannitol-treated animals (Fig. 3).

Urine flow rate during the baseline control period

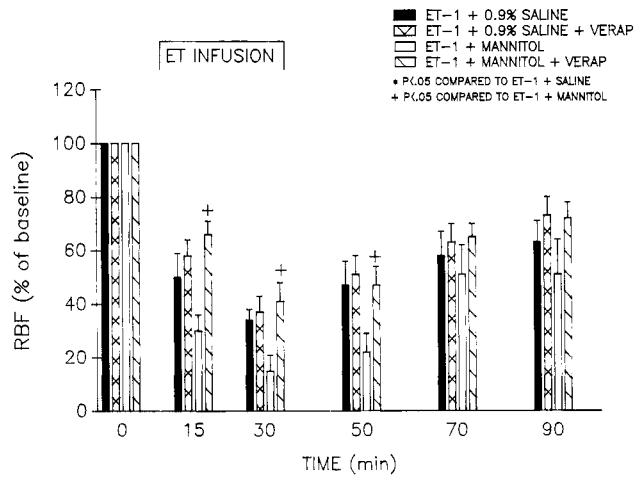

Figure 3. RBF values (percentage of baseline) are shown before, during, and after intravenous infusions of endothelin (110 ng kg^{-1} min^{-1}) into saline (0.5 ml kg^{-1} min^{-1})-expanded rats (Group 1, solid bars), into rats treated with saline + verapamil (0.02 mg kg^{-1} min^{-1}) (Group 2, cross-hatched bars), into rats treated with 10% mannitol in saline (0.5 ml kg^{-1} min^{-1}) (Group 3, open bars), and into rats treated with 10% mannitol in saline + verapamil (Group 4, right-slanted bars). *$P < 0.05$ compared with saline alone; +, $P < 0.05$ compared with 10% mannitol alone. Statistical evaluation of time-related changes in RBF are reported in Table I.

was significantly greater in mannitol-treated rats compared with saline-expanded animals (200 ± 17 μl/min vs 79 ± 12 μl/min, respectively). However, the fractional decrease in urine flow rate in the saline group at 30 min during the infusion of endothelin (43 ± 8% of the baseline value) was not significantly different from the mannitol group (34 ± 3% of the baseline value). By contrast, verapamil significantly attenuated the endothelin-induced decrease in urine flow rate in mannitol-treated rats (urine flow rate decreased to only 65 ± 6% of the baseline value; $P < 0.05$ compared with mannitol alone), but had no effect on the fractional decrease in urine flow rate prompted by the infusion of the peptide into saline-expanded rats (urine flow rate decreased to 40 ± 7% of the baseline value; $P < 0.05$ compared with mannitol + verapamil, but not significantly different from saline alone).

Discussion

The results of the present study demonstrate that volume expansion, either with saline or with mannitol, does not alter the systemic or renal vasoconstriction induced by an intravenous infusion of endothelin. Volume expansion with mannitol, but not with saline, does, however, change the apparent dependence on extracellular Ca^{2+} of the renal vasoconstriction prompted by the peptide. Thus, during volume expansion with mannitol, the endothelin-induced renal vasoconstriction was significantly reduced by verapamil, whereas the renal actions of endothelin were relatively unaffected by the calcium channel antagonist in saline-expanded rats. Finally, the systemic action of endothelin, as defined by the increase in mean arterial blood pressure, was attenuated by verapamil during saline and mannitol expansion, indicating that a significant component of the systemic response to the peptide under both conditions is dependent on extracellular Ca^{2+} influx.

There have been conflicting reports with regard to effects of Ca^{2+} channel antagonists on the systemic versus renal actions of endothelin (20–22). We reported previously that the endothelin-induced increase in mean arterial blood pressure in non-volume-expanded rats is virtually abolished by verapamil and by Mn^{2+}, whereas the decrease in GFR is not affected by either of those agents (20). In that study, we also reported that verapamil had no effect on the endothelin-induced decrease in renal blood flow (measured with a flow probe) in the dog (20). Madeddu *et al.* (21) have reported that, in conscious rats, both the systemic and renal vasoconstriction prompted by an intravenous bolus injection of endothelin are attenuated by nifedipine but that, compared with the systemic circulation, the renal circulation is not nearly as sensitive to the antagonist. Katoh *et al.* (22) reported that the endothelin-induced renal vasoconstriction is significantly attenuated by nicardipine when both endothelin and the Ca^{2+} channel antagonist are infused directly into the renal artery of the rat. However, in the latter study (22), the investigators did not report the effects of nicardipine alone on renal blood flow prior to infusing endothelin. Given the well-known renal vasodilation associated with intrarenal infusions of Ca^{2+} channel antagonists (see, for example, Fig. 5 in Ref. 20), it is possible that a significant portion of the attenuation reported by Katoh *et al.* (22) was related to an offsetting renal vasodilation associated with the nicardipine infusion.

One of the interesting facets of the current study relates to the fact that the renal actions of endothelin were attenuated by verapamil in mannitol-expanded rats, but not in saline-expanded rats. Endothelin elicits a contraction of both the afferent and efferent arteriole (9, 10). However, the endothelin-induced contraction of only the afferent arteriole is attenuated by verapamil; endothelin-induced contractions of the efferent arteriole are not affected by the Ca^{2+} channel antagonist (9, 10). Therefore, one interpretation of the current data is that in saline-expanded animals treated with verapamil, endothelin-induced decreases in the GFR and RBF may be due primarily to an increase in efferent arteriolar resistance. On the other hand, in rats treated with verapamil and mannitol, the peptide-induced decreases in RBF and GFR are attenuated, indicating that under these conditions the primary site of action of endothelin could be in the afferent arteriole. Clearly, however, additional studies will be required to explain the mechanism by which mannitol and/or the relative distribution of fluid between the intracellular and extracellular

fluid spaces alters the apparent extracellular Ca^{2+} requirements of the endothelin-induced renal vasoconstriction.

In summary, volume expansion with either saline or with mannitol does not alter systemic or renal vasoconstriction associated with a constant infusion of endothelin. Moreover, the endothelin-induced increase in mean arterial blood pressure during expansion with either agent is significantly attenuated by verapamil, indicating a major role for extracellular Ca^{2+} influx in that event. By contrast, the renal vasoconstriction prompted by the peptide is not affected by verapamil during saline expansion, whereas it is significantly attenuated during volume expansion with mannitol. These data suggest that during volume expansion with mannitol, but not with saline, the ET-induced renal vasoconstriction occurs primarily at intrarenal resistance sites that are dependent upon extracellular Ca^{2+}.

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