Lead and Nickel Alter the Cardiorenal Actions of Endothelin in the Rat (43851)

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Abstract. **In the current study, we have determined to what extent lead and nickel alter the cardiorenal actions of endothelin in pentobarbital anesthetized female rats. One hour following surgery, 3 × 15-min renal clearances were collected and endothelin (ET)-1 was infused iv at 110 ng/kg/min for 30 min during which time an additional two clearances were collected. Lead (infused as lead acetate throughout the experiment) at 4.8 nmoles/min and 24 nmoles/min significantly attenuated the ET-induced increase in mean arterial pressure (MAP); lead infused at 0.48 nmoles/min had no effect. An ET-induced decrease in the glomerular filtration rate (GFR) in control rats was completely blocked by the higher doses of Pb^{2+}. By contrast, Pb^{2+} had no effect on angiotensin II or norepinephrine induced increases in MAP. In additional experiments, calcium chloride was infused at 500 nmoles/min for 105 min, then Ca^{2+} + Pb^{2+} (4.8 nmoles/min) were infused for another 105 min; in these experiments, there was no Pb^{2+}-induced inhibition of the MAP response to endothelin; the GFR response to the peptide remained blocked. $NiCl_2$ reduced the ET-induced increase in MAP only at 24 nmoles/min; at 4.8 and 24 nmoles/min, nickel attentuated the decrease in GFR induced by ET. Finally, Ca^{2+} infusion had no effect on the inhibition by Ni^{2+} of the GFR response to ET. These data illustrate that (i) lead inhibits the cardiorenal actions of endothelin; (ii) a Ca^{2+}-related process is involved the systemic but not the renal component of this inhibition; (iii) since the heavy metal does not affect angiotensin II or norepinephrine-induced increases in MAP, the inhibition by lead of the systemic response is relatively specific for endothelin; and (iv) nickel also inhibits the renal response to the peptide but higher doses are required to inhibit the systemic response.** [P.S.E.B.M. 1995, Vol 208]

Endothelin (ET) is a 21 amino acid vasoactive peptide initially isolated from endothelial cells (1). The cardiovascular actions of this peptide include sustained increases in mean arterial pressure, positive inotropic and chronotropic effects on the heart, and vasoconstriction of most arterial components (2, 3). Endothelin also stimulates the secretion of atrial natriuretic peptide, renin, and aldosterone (2, 3). The actions of the peptide on the kidney include increases in renal vascular resistance and concomitant decreases in renal blood flow, the glomerular filtration rate (GFR), and the ultrafiltration coefficient, and variable effects on sodium and water reabsorption (2–5).

Simonson and Dunn (6) have recently reviewed the mechanism of endothelin-induced vasoconstriction; at least two intracellular signal transduction pathways appear to be involved. Thus, the vascular response to endothelin is biphasic, with an initial transient increase in intracellular calcium, a process which is mediated through an increase in inositol trisphosphate (IP_3) (7). The second phase is characterized by a sustained increase in intracellular calcium that is due to the entry of extracellular calcium ions (7, 8). Endothelin may activate a receptor-operated, voltage insensitive calcium channel (9) as well as an L-type calcium channel (10).

The degree to which the ET-induced vasoconstriction is dependent on extracellular calcium varies among tissue types. Banks and Cao (4) reported that the L-

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type calcium channel blocker, verapamil, abolishes the ET-induced increase in mean arterial pressure, but does not alter the endothelin-induced decreases in glomerular filtration rate. Along these lines, Edwards *et al.* (11) and Loutzenhiser *et al.* (12) reported that endothelin-induced contractions of the afferent but not the efferent arteriole are attentuated by calcium channel antagonists. In addition, *in vitro* studies have shown that dihydropyridine calcium channel antagonists inhibit the ET-induced vasoconstriction in porcine coronary arteries by preventing the sustained increase in intracellular calcium (10). Similarly, in the guinea pig trachea the responses to endothelin are dependent on extracellular calcium (13). By contrast, neither nicardipine nor removal of extracellular calcium completely abolishes the ET-induced contraction of the rabbit aorta (14). Shimamoto *et al.* (15) reported similar findings in the rat aorta; only a small component of the endothelin-induced response is dependent on extracellular calcium entry through voltage dependent calcium channels. Finally, Tanoi *et al.* (16) reported that the calcium channel blocker, nifedipine, inhibits the endothelin-induced contractions in the cerebral and coronary arteries but not in mesenteric arteries from the dog.

Heavy metals such as cadmium and nickel have been shown to alter the vasoconstrictor responses to endothelin-1. *In vitro* studies with nickel and cadmium have shown that these heavy metals inhibit the ET-induced vasoconstriction in isolated arterial strips (17, 18). Despite the *in vitro* findings there have been no *in vivo* studies on the effects of heavy metals on the cardiorenal actions of endothelin-1. Lead has been reported to be a calcium channel blocker which is effective in blocking both L- and t-type Ca^{2+} channels (19); however, its effects on the cardiorenal actions of endothelin have not been evaluated. Therefore, in the current study we have examined the effects of lead and nickel on endothelin-induced changes in mean arterial pressure and renal function, *in vivo,* utilizing anesthesized Sprague-Dawley rats.

Materials and Methods

Ninety-two female Sprague-Dawley rats (200 to 250 g) were maintained on standard rat chow and water *ad libitum* until the time of the experiment. Rats were anesthetized with sodium pentobarbital (60 mg/kg, ip) and rectal temperatures were 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 pressures were 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 deliver saline with lead acetate, saline with sodium acetate (control for lead experiments), saline with nickel chloride or saline (control for nickel chloride) via the left femoral vein at a rate of 50 μl/min. Finally, the bladder was cannulated with PE-100 tubing via an abdominal incision. Following surgery, the animal was placed on its side slightly elevated above the table to prevent accumulation of urine in the bladder. Upon completion of each experiment all rats were sacrificed with a lethal dose of sodium pentobarbital.

Five series of experiments with a total of eight groups of rats were conducted. Experiments in Series I were divided into four groups of rats (n = 8 per group) and were designed to determine to what extent lead altered the cardiorenal actions of endothelin: Group I-I was a control group and received 4.8 nmoles/min sodium acetate, Group I-II received 0.48 nmoles/min lead acetate (PbAc), Group I-III received 4.8 nmoles/min PbAc, and Group I-IV received 24 nmoles/min PbAc. The doses of lead were chosen experimentally; after determining a dose which altered the cardiorenal actions of endothelin-1, lower and higher doses were then evaluated. In each experiment, NaAc or PbAc solutions were infused throughout the entire experiment. Rats were administered 1.3 mg of sodium pentobarbital following surgery and again 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_2, and C_3. Endothelin-1 was then added to the creatinine infusion to yield a dose of 110 ng/kg/min; the dose of endothelin was chosen because it produces near maximal effects on the mean arterial pressure and renal function (2, 20). Endothelin was infused for 30 min during which time 2 × 15-min clearances (E_1 and E_2) were collected; a second arterial blood sample (0.5 ml) was obtained immediately following E_2.

Experiments in Series II were designed to evaluate the actions of a second heavy metal, nickel, on the cardiorenal actions of endothelin; there were a total of four groups of rats in Series II. Group II-I (n = 8) was a control group and received only saline throughout the experiment, Group II-II (n = 5 for this and all other groups in Series II) received 0.48 nmoles/min $NiCl_2$, Group II-III received 4.8 nmoles/min $NiCl_2$, and Group II-IV received 24 nmoles/min $NiCl_2$ (i.e., equivalent doses of nickel with that of lead used in Series I). The experimental protocol was also the same as in Series I.

Experiments in Series III (n = 5) were designed to evaluate to what extent calcium was involved in lead-induced changes in the cardiorenal actions of endothelin. Following surgery, calcium chloride was infused at 500 nmoles/min for 60 min. PbAc (4.8 nmoles/min)

plus 500 nmoles/min $CaCl_2$ was then infused for 60 min followed by 3 × 15-min control periods (C_1, C_2 and C_3) with a blood sample of 0.2 ml taken after C_1. The remainder of the experimental protocol was the same as Series I.

Experiments in Series IV (n = 5) were similar to those in Series III and were designed to evaluate to what extent calcium was involved in nickel-induced changes in the cardiorenal actions of endothelin. The experimental protocol for this series of experiments was the same as for Series III except that $NiCl_2$ (4.8 nmoles/min) was infused.

Two groups of rats (n = 8/group) were evaluated in Series V; these experiments were designed to evaluate the specificity of lead-induced changes on the cardiorenal actions for endothelin. Experiments in this series were performed with angiotensin II (Ang II) (50 ng/kg/min, Group V-I) and with norepinephrine (NE) (1 μg/kg/min, Group V-II). The doses of Ang II and NE were chosen to induce increases in MAP similar in magnitude to those prompted by endothelin in the Series I experiments. In group V-I, 2 × 15-min clearance periods (C_1 and C_2) were performed and a 0.2-ml blood sample obtained after C_1. Ang II was infused for 30 min during which time 2 × 15-min clearances were collected (E_1 and E_2); an arterial blood sample of 0.2 ml was obtained after E_2. Next, 4.8 nmoles/min PbAc was infused for 75 min with additional doses of sodium pentobarbital administered at 20 and 40 min during the infusion of PbAc. Two × 15-min clearances were collected (Pb_1 and Pb_2) and a 0.2-ml arterial sample withdrawn after Pb_1. PbAc infusion was continued but, in addition, Ang II was then infused for 30 min with 2 × 15-min clearances being collected (PE_1 and PE_2); a 0.5-ml blood sample was collected after PE_2. A similar protocol was used for Group V-II (the NE group) except that two subgroups of animals (V-II_A and V-II_B) were required because the blood pressures in several animals following the first NE infusion were below 80 mm Hg. In Subgroup V-II_A, only NaAc was infused (as described above for Group V-I), and the experiment was terminated after the infusion of NE. In Subgroup V-II_B, PbAc was infused for 90 min followed by an infusion of NE.

Chemical Determinations. Urine volume (V) was determined gravimetrically. Creatinine concentrations in urine and blood were measured by the method of Folin and Wu (21); female rats were chosen since the clearance of creatinine can be equated with the glomerular filtration rate (GFR) (22). Urinary and plasma sodium (Na^+) and the total plasma calcium (Ca^{2+}) were determined by atomic emission and absorption spectrophotometry, respectively, using a Perkin-Elmer atomic absorption spectrophotometer.

Endothelin-1 was purchased from Peninsula Laboratories and was dissolved in a solution of 0.1% acetic acid and 0.01% Triton 305, divided into aliquots and stored at −80°C until the time of 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

Lead- and Nickel-Induced Changes in the Systemic Response to ET. The effects of lead (Series I) and nickel (Series II) infused intravenously at 0.48 nmoles/min, 4.8 nmoles/min, and 24 nmoles/min on the cardiorenal actions of endothelin are summarized in Table I. There were no significant differences in baseline mean arterial blood pressure values among the groups of rats in Series I and Series II. In all the groups, there were significant increases, compared with baseline, in mean arterial blood pressure (MAP) at 15 and 30 min during infusion of endothelin. However, MAP values during infusion of endothelin in rats treated with 4.8 and 24 nmoles/min PbAc were significantly lower than corresponding MAP values in rats treated with NaAc or with 0.48 nmoles/min PbAc. By contrast, during infusion of endothelin in Series II rats, MAP values for the nickel-treated animals were not significantly different from the saline-treated rats except at the 30-min time period for the group treated with 24 nmoles/min $NiCl_2$ (Group II-IV).

Further analysis of the effects of lead and nickel on endothelin-induced increases in mean arterial blood pressure are illustrated in Figures 1 and 2. These figures illustrate MAP data expressed as the change in pressure (Δmm Hg) during infusion of endothelin at 15 and 30 min. The increases in mean arterial blood pressure at 30 min during infusion of endothelin were not significantly different between control (NaAc) rats (35 ± 4 mm Hg) and rats treated with 0.48 nmoles/min PbAc (29 ± 4 mm Hg). By contrast, in rats infused with the higher doses of lead (4.8 and 24 nmoles/min PbAc) the increases in MAP (16 ± 6 and 16 ± 3 mm Hg, respectively) were significantly less than the increases in the MAP recorded in controls. In nickel-treated rats (Fig. 2), increases in mean arterial pressure at 30 min during infusion of endothelin were not significantly different between control rats (44 ± 4 mm Hg) and rats treated with 0.48 nmoles/min $NiCl_2$ (41 ± 8 mm Hg) and 4.8 nmoles/min $NiCl_2$ (32 ± 6 mm Hg). In rats infused with the highest dose of nickel (24 nmoles/min $NiCl_2$), the ΔMAP values were significantly lower than the control group (the change in MAP in Group II-IV was 26 ± 5 mm Hg at 30 min).

Table I. Effects of Lead and Nickel on Endothelin-Induced Changes in Mean Arterial Blood Pressure and Selected Variables of Renal Function[a]

LEAD				NICKEL			
Series I	Baseline	ET INFUSION		Series II	Baseline	ET INFUSION	
		15 min	30 min			15 min	30 min
Group I–I (4.8 nmoles/min NaAc) (n = 8)				Group II–I (Saline) (n = 8)			
MAP (mmHg)	95 ± 4	117 ± 4**	130 ± 4**	MAP (mmHg)	94 ± 4	124 ± 6**	139 ± 5**
GFR (ml/min)	3.05 ± 0.26	1.85 ± 0.24**	1.28 ± 0.29**	GFR (ml/min)	2.91 ± 0.30	1.50 ± 0.18**	0.52 ± 0.17**
$U_{Na}V$ (μEq/min)	3.52 ± 1.0	9.28 ± 2.2	6.02 ± 1.6	$U_{Na}V$ (μEq/min)	4.05 ± 0.74	7.53 ± 1.5**	2.16 ± 0.59
V (μl/min)	43 ± 8	68 ± 13	53 ± 11	V (μL/min)	37.2 ± 2.2	53.4 ± 7.6*	19.1 ± 5.56*
FE_{Na} (%)	0.87 ± 0.22	3.99 ± 0.81**	4.11 ± 0.55**	FE_{Na} (%)	1.12 ± 0.19	4.37 ± 0.88**	4.07 ± 0.56**
Group I–II (0.48 nmoles/min PbAc) (n = 8)				Group II–II (0.48 nmoles/min $NiCl_2$) (n = 5)			
MAP (mmHg)	100 ± 4	117 ± 5**	130 ± 4**	MAP (mmHg)	94 ± 5	122 ± 8**	135 ± 7**
GFR (ml/min)	2.57 ± 0.21	2.01 ± 0.25*	1.67 ± 0.50*	GFR (ml/min)	2.82 ± 0.19	1.03 ± 0.17*	0.32 ± 0.14*
$U_{Na}V$ (μEq/min)	3.91 ± 1.2	6.79 ± 1.7	5.66 ± 1.9	$U_{Na}V$ (μEq/min)	3.76 ± 1.05	7.08 ± 2.91	1.20 ± 0.66
V (μl/min)	35 ± 8	50 ± 10	36 ± 10	V (μl/min)	26.2 ± 7.80	26.9 ± 8.00	6.98 ± 2.74*
FE_{Na} (%)	0.9 ± 0.28	2.15 ± 0.67**	2.12 ± 0.46**	FE_{Na} (%)	0.72 ± 0.20	3.12 ± 1.08	4.39 ± 2.18
Group I–III (4.8 nmoles/min PbAc) (n = 8)				Group II–III (4.8 nmoles/min $NiCl_2$) (n = 5)			
MAP (mmHg)	89 ± 3	101 ± 6**	107 ± 7**	MAP (mmHg)	94 ± 4	112 ± 6*	127 ± 7**
GFR (ml/min)	2.35 ± 0.27	2.31 ± 0.35	2.18 ± 0.31	GFR (ml/min)	3.25 ± 0.13	2.70 ± 0.27*	2.37 ± 0.16**
$U_{Na}V$ (μEq/min)	2.89 ± 0.46	5.03 ± 1.2*	7.05 ± 1.4**	$U_{Na}V$ (μEq/min)	8.08 ± 1.30	12.5 ± 1.84*	15.4 ± 1.36**
V (μl/min)	36 ± 8	38 ± 7	48 ± 9	V (μl/min)	54.0 ± 7.2	64.7 ± 9.9	82.5 ± 10.4
FE_{Na} (%)	1.18 ± 0.37	1.85 ± 0.76	2.81 ± 1.08*	FE_{Na} (%)	1.55 ± 0.37	2.54 ± 0.37*	3.55 ± 0.43*
Group I–IV (24 nmoles/min/PbAc) (n = 8)				Group II–IV (24 nmoles/min $NiCl_2$) (n = 5)			
MAP (mmHg)	96 ± 4	103 ± 5**	112 ± 5**	MAP (mmHg)	105 ± 5	121 ± 5**	131 ± 6**
GFR (ml/min)	2.89 ± 0.26	2.59 ± 0.18	2.52 ± 0.20	GFR (ml/min)	2.91 ± 0.09	2.91 ± 0.37	2.44 ± 0.39
$U_{Na}V$ (μEq/min)	1.75 ± 0.6	4.34 ± 1.5*	6.26 ± 2.3*	$U_{Na}V$ (μEq/min)	4.10 ± 2.12	8.76 ± 4.36	3.51 ± 0.6
V (μl/min)	18 ± 4	29 ± 10*	38 ± 10*	V (μl/min)	36.1 ± 15.1	47.7 ± 22.0	24.5 ± 4.8
FE_{Na} (%)	0.34 ± 0.08	0.87 ± 0.27*	1.39 ± 0.49**	FE_{Na} (%)	1.35 ± 0.59	2.38 ± 0.92	1.48 ± 0.23
Series III ($PbAc + CaCl_2$) (n = 8)				Series IV ($NiCl_2 + CaCl_2$) (n = 5)			
MAP (mmHg)	92 ± 3	108 ± 3**	123 ± 4**	MAP (mmHg)	97 ± 8	133 ± 9**	136 ± 9**
GFR (ml/min)	2.77 ± 0.31	2.39 ± 0.11	2.22 ± 0.23	GFR (ml/min)	2.94 ± 0.57	2.40 ± 0.29	1.57 ± 0.10*
$U_{Na}V$ (μEq/min)	8.70 ± 1.3	9.80 ± 1.4	10.0 ± 1.6	$U_{Na}V$ (μEq/min)	7.40 ± 1.4	8.60 ± 0.60	5.1 ± 0.8
V (μl/min)	52 ± 10	63 ± 14*	71 ± 14**	V (μl/min)	72 ± 12	87 ± 14	67 ± 15
FE_{Na} (%)	2.98 ± 0.62	2.48 ± 0.37	3.01 ± 0.69	FE_{Na} (%)	3.4 ± 0.70	3.9 ± 0.50	3.60 ± 0.70

[a] Values are means ± SE. GFR = glomerular filtration rate; $U_{Na}V$ = urinary sodium excretion; V = urine flow rate; FE_{Na} = fractional sodium excretion.
* $P < 0.05$ compared with corresponding baseline value.
** $P < 0.01$ compared with corresponding baseline value.

Lead- and Nickel-Induced Effects on the Renal Response to ET. Table I also summarizes the effects of lead and nickel on endothelin-induced changes in renal function. As was observed with mean arterial blood pressure values, there were no significant differences in the baseline GFR values among the eight groups of rats in Series I and II. However, during the infusion of endothelin, rats treated with NaAc (Group I-I) as well as rats treated with 0.48 nmoles/min PbAc (Group I-II) were characterized by significant decreases in the GFR when compared with the baseline value for each group. By contrast, in rats treated with either 4.8 nmoles/min PbAc (Group I-III) or 24 nmoles/min PbAc (Group I-IV), there were no significant endothelin-induced decreases in the GFR. Similarly, during infusion of endothelin into the rats treated with saline (Group II-I), 0.48 nmoles/min $NiCl_2$ (Group II-II), or 4.8 nmoles/min $NiCl_2$ (Group II-III) there were significant decreases in the GFR when compared with the baseline GFR value for each group. By contrast, in rats treated with 24 nmoles/min $NiCl_2$, there was no significant endothelin-induced decrease in the GFR. When compared with the saline controls, the GFR values of the rats treated with either 4.8 nmoles/min $NiCl_2$ or 24 nmoles/min $NiCl_2$ were significantly higher at both 15 and 30 min during infusion of endothelin.

Figures 3 and 4 illustrate the GFR data when expressed as the endothelin-induced change in this physiological variable (Δml/min). In rats infused with 4.8 nmoles/min NaAc or with 0.48 nmoles/min PbAc, endothelin induced significant decreases in the GFR; the GFR decreased 1.77 ± 0.38 ml/min and 0.89 ± 0.34 ml/min in the two groups, respectively. As summarized in Table I, the decrease in GFR prompted by endothelin was completely blocked in rats infused with either 4.8 or 24 nmoles/min PbAc.

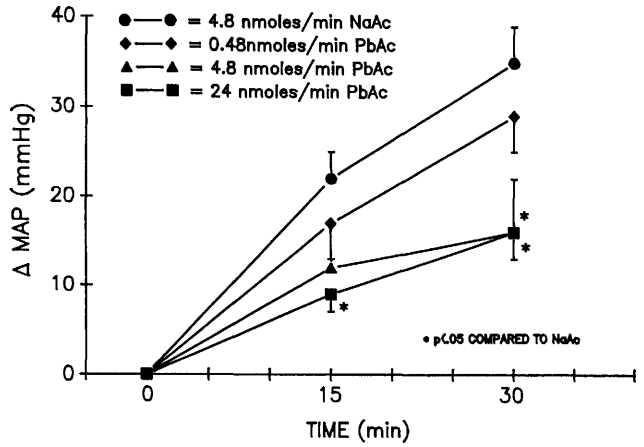

Figure 1. Changes in mean arterial blood pressure values (ΔMAP, mm Hg) are shown during intravenous infusions of endothelin-1 (ET) (110 ng/kg/min) into rats treated with 4.8 nmoles/min NaAc (Group I-I, circles), into rats treated with 0.48 nmoles/min PbAc (Group I-II, diamonds), into rats treated with 4.8 nmoles/min PbAc (Group I-III, triangles), and into rats treated with 24 nmoles/min PbAc (Group I-IV, squares). $^*P < 0.05$ compared with corresponding control values in NaAc (Group I-I) treated rats. Statistical evaluation of values relative to baseline are reported in Table I.

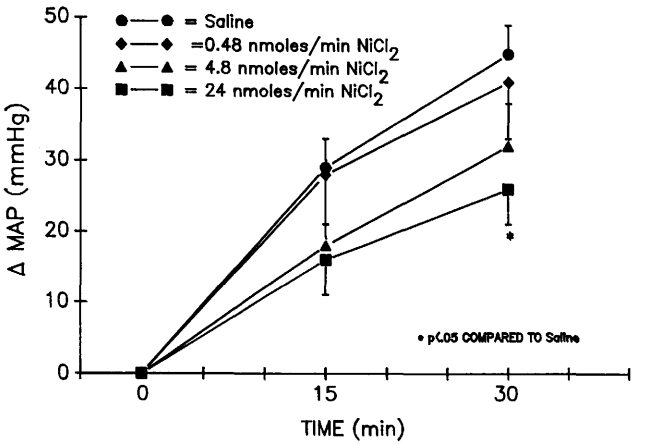

Figure 2. Changes in mean arterial blood pressure values (ΔMAP, mm Hg) are shown during intravenous infusions of endothelin-1 (ET, 110 ng/kg/min) into rats treated with saline (Group II-I, circles), into rats treated with 0.48 nmoles/min $NiCl_2$ (Group II-II, diamonds), into rats treated with 4.8 nmoles/min $NiCl_2$ (Group II-III, triangles), and into rats treated with 24 nmoles/min $NiCl_2$ (Group II-IV, squares). $^*P < 0.05$ compared with corresponding control values in saline (Group II-I) treated rats. Statistical evaluation of values relative to baseline are reported in Table I.

In rats infused with saline or with 0.48 nmoles/min $NiCl_2$ (Fig. 4), endothelin induced significant decreases in the GFR; the GFR decreased 2.39 ± 0.38 ml/min and 2.5 ± 0.33 ml/min, in the two groups, respectively. By contrast, the GFR decreased only 0.88 ± 0.21 ml/min in the group receiving 4.8 nmoles/min $NiCl_2$ and 0.46 ± 0.43 ml/min in the group receiving 24 nmoles/min $NiCl_2$.

Table I also summarizes data for urinary sodium excretion rate ($U_{Na}V$), urinary flow rate (V), and fractional sodium excretion ($FE_{Na} = U_{Na}V/GFR \times$

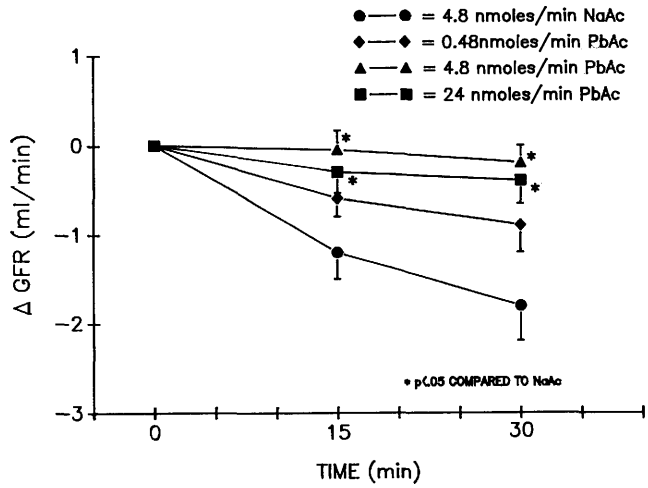

Figure 3. Changes in glomerular filtration rates (ΔGFR, ml/min) are shown during intravenous infusions of endothelin-1 (ET, 110 ng/kg/min) into rats treated with 4.8 nmoles/min NaAc (Group I-I), into rats treated with 0.48 nmoles/min PbAc (Group I-II), into rats treated with 4.8 nmoles/min PbAc (Group I-III), and into rats treated with 24 nmoles/min PbAc (Group I-IV). See legend to Figure 1 for description of symbols. $^*P < 0.05$ compared with corresponding values in the appropriate control group, NaAc (Group I-I). Statistical evaluation of values relative to baseline are reported in Table I.

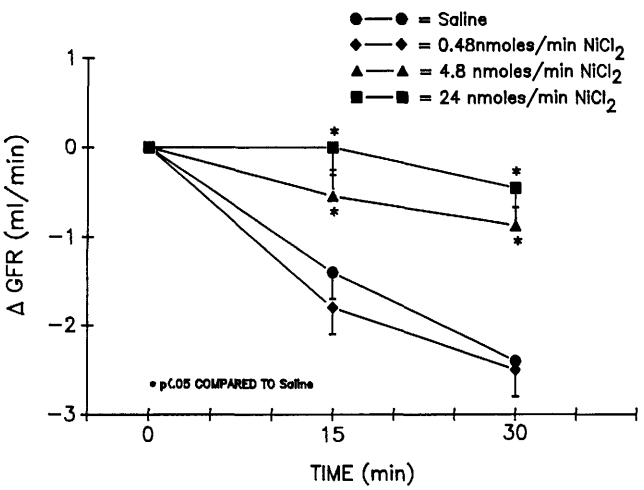

Figure 4. Changes in glomerular filtration rates (ΔGFR, ml/min) are shown during intravenous infusions of endothelin-1 (ET, 110 ng/kg/min) into rats treated with saline (Group II-I), into rats treated with 0.48 nmoles/min $NiCl_2$ (Group II-II), into rats treated with 4.8 nmoles/min $NiCl_2$ (Group II-III), and into rats treated with 24 nmoles/min $NiCl_2$ (Group II-IV). See legend to Figure 1 for description of symbols. $^*P < 0.05$ compared with corresponding values in the appropriate control group, saline (Group II-I). Statistical evaluation of values relative to baseline are reported in Table I.

plasma Na concentration). Rats administered 4.8 and 24 nmoles/min PbAc were characterized by significant increases in $U_{Na}V$ when compared with corresponding baseline values. In addition, rats infused with 24 nmoles/min PbAc had significant increases in V at 15 and 30 min during infusion of ET. Fractional excretion of sodium increased significantly in the NaAc group and in all three of the lead-treated groups compared to their

corresponding baselines. In the saline-treated controls (Group II-I), V and FE_{Na} were significantly different from baseline at both 15 and 30 min whereas $U_{Na}V$ was only significantly higher than baseline at 15 min. In rats infused with 0.48 nmoles/min $NiCl_2$, the only statistically significant change was in V, which was significantly lower than baseline at 30 min. By contrast, in rats receiving 4.8 nmoles/min $NiCl_2$, $U_{Na}V$ and FE_{Na} values were significantly above baseline at both 15 and 30 min; V was significantly higher than baseline at 30 min.

Series III and IV: Calcium Infusion Experiments. Table I summarizes the data from the calcium infusion experiments. Treatment with calcium (500 nmoles/min Ca^{2+} for 105 min followed by Ca^{2+} + 4.8 nmoles/min PbAc for 105 min, Series III) prevented the lead-induced inhibition of the systemic response to endothelin. The MAP values were not significantly different from the NaAc group (Group I-I). By contrast, the GFR response to endothelin remained attentuated in calcium + lead treated rats. GFR values at 15 and 30 min during endothelin infusion were not significantly different from baseline in this group of rats. It is also of interest to note that the infusion of calcium chloride did not alter the plasma calcium concentration. Specifically, control rats had a mean total plasma calcium concentration of 5.1 ± 0.3 mEq/l, a value that was not significantly different from the total plasma calcium concentration of the calcium-treated group (4.26 ± 0.2 mEq/l).

In Series IV, treatment with calcium (500 nmoles/min Ca^{2+} for 105 min followed by Ca^{2+} + 4.8 nmoles/min $NiCl_2$ for 105 min) did not affect the ET-induced increases in MAP (data also shown in Table I). The inhibition by nickel of the endothelin-induced decreases in GFR was also unaffected by treatment with calcium.

Effects of Lead on the Systemic Response to ANG II and NE. The effects of PbAc infused at 4.8 nmoles/min on the cardiorenal actions of ANG II (Group V-I) and NE (Group V-II) are summarized in Table II. Both Ang II and NE produced increases in MAP during infusion that were not significantly different with either NaAc or PbAc. The doses of Ang II and NE used in the current study did not affect the GFR in rats infused with either NaAc or PbAc.

Discussion

The results of the current study demonstrate that intravenous infusions of lead inhibit both the increase in mean arterial blood pressure and the decrease in glomerular filtration rate induced by endothelin. Treatment with calcium blocked the lead-induced inhibition of the MAP but not the GFR response to the peptide. Moreover, the effects of lead appear to be specific for endothelin as there were no effects of lead on Ang II- or norepinephrine-induced increases in mean arterial blood pressure. Intravenous infusions of nickel inhibit the renal response to endothelin and, at higher doses, the systemic response to the peptide. As with lead, exogenously administered calcium had no effect on the inhibition of the renal response to ET-1 by nickel.

Lead and nickel may alter the cardiorenal actions of endothelin by a number of mechanisms including inhibition of the interaction between endothelin and its receptor(s), inhibition of intracellular signaling pathways and/or the release of intracellular (or influx of extracellular) calcium.

The fact that nickel inhibits the renal effects of endothelin-1 at doses that are lower than are needed to inhibit the systemic response whereas both the systemic and renal actions of the peptide are attentuated by the same dose of lead is an interesting facet of the current study. Since there are at least two ET receptor subtypes, ET_A and ET_B, our data could be explained by a differential sensitivity of the endothelin subtypes to the heavy metals. Functional studies have shown that ET-induced increases in mean arterial blood pressure are mediated by ET_A receptors whereas non-ET_A receptors are involved in ET-induced renal vasocon-

Table II. Effects of Lead on the Cardiorenal Actions of Angiotensin II and Norepinephrine[a]

	MAP (mm Hg)		GFR (ml/min)	
	Basal	30 min with agonist	Basal	30 min with agonist
Group V-I (ANG II)				
4.8 NaAc	90 ± 4	119 ± 4**	2.30 ± 0.25	3.09 ± 0.66
4.8 PbAc	89 ± 4	123 ± 7**	2.69 ± 0.24	2.46 ± 0.25
Group V-II_A (NE)				
4.8 NaAc	86 ± 2	113 ± 5**	2.34 ± 0.06	2.62 ± 0.24
Group V-II_B (NE)				
4.8 PbAc	85 ± 2	110 ± 5**	3.05 ± 0.12	2.31 ± 0.34

[a] Values are means ± SE; amounts of lead acetate or sodium acetate are in nmoles per min (Ang II experiments were performed in the same rats before and during PbAc (n = 8) whereas NE experiments were performed in two separate subgroups of eight rats per sub-group).
* $P < 0.05$ compared with corresponding baseline value.
** $P < 0.01$ compared with corresponding baseline value.

striction (24). Furthermore, Biguad and Pelton have reported that an ET_B agonist [$Ala^{1,3,11,15}$] ET-1 induced an initial decrease in arterial pressure followed by mesenteric and renal vasoconstriction (25). Along these lines, it is of interest to note that Wada *et al.* (23) reported that nickel and cadmium inhibit the binding of endothelin to its receptor; the differential effects of the heavy metals on ET receptor subtypes were not evaluated since the Wada *et al.* report (23) was published prior to the description of multiple ET receptors.

The possibility that lead may interfere with an intracellular signaling pathway necessary for vascular smooth muscle contraction does not appear to be supported by the data from the current study. It is generally held that endothelin, norepinephrine, and angiotensin II utilize similar transduction pathways with inositol triphosphate (IP_3) as an important second messenger stimulating the release of intracellular Ca^{2+} pools (for reviews see Ref. 6, 26, and 27). Nonetheless lead did not block the norepinephrine or angiotensin II-induced increase in mean arterial blood pressure, data which suggest that the heavy metal does not block an IP_3 signaling pathway. Other investigators have also shown that contractile responses to angiotensin II and norepinephrine are not inhibited by lead but are either enhanced or unchanged by the heavy metal (28). Thus, the lead-induced inhibition of the cardiorenal actions of endothelin appears to be relatively specific for endothelin-1 and is not a general phenomenon associated with all vasoconstrictor agents. The possibility does exist that endothelin may utilize other intracellular signaling pathways such as protein kinase C or phospholipase D (see review by Masaki *et al.* [29]) which could be sensitive to inhibition by lead. Clearly, additional studies will be required to further evaluate possible intracellular mechanisms by which lead could inhibit the cardiorenal actions of endothelin.

It is of interest that pretreatment with calcium offsets the inhibition by lead of the systemic response to endothelin. Although there was no change in the concentration of calcium in these experiment, these data suggest that lead might interfere with the influx of extracellular calcium in those vessels responsible for the increase in mean arterial blood pressure. Along these lines, it has been reported that ET acts directly or indirectly by activating L-type calcium channels (7). Moreover, the systemic actions of endothelin, as reflected by the increase in arterial blood pressure, are verapamil sensitive whereas the renal actions of the peptide are not affected by the calcium channel antagonist (4, 29). It is of interest to note that lead blocks L-type voltage gated channels with an IC_{50} of less than 1.0 μM (19) whereas very high concentrations (5 mM) of nickel are required to block L-channels (30). Thus, the fact that lead inhibits the systemic response to endothelin at doses lower than does nickel may be related to an effect of the heavy metal on L-type calcium channels. Since the renal response to endothelin is not verapamil sensitive (4) the inhibition by lead and nickel of the renal response cannot be explained simply by the blockade of L-type calcium channels. Perhaps the inhibition of the renal response involves the interaction with another type of calcium channel. Along these lines, lead and nickel do block t-type calcium channels with an IC_{50} of 1.3 μM (19) and 47 μM (31), respectively. Therefore, it is possible that both lead and nickel alter the renal response to endothelin by inhibiting t-channel activity. In fact, Furukawa *et al.* (32) have reported that endothelin enhances calcium entry through t-type calcium channels in neonatal rat ventricular myocytes. The role of t-type calcium channels in other tissues has not been widely studied but there have been a few reports that nickel inhibits endothelin-induced contractions in isolated vessels (17, 33).

It has been reported that endothelin, in addition to causing a decrease in the GFR, has a number effects on renal function. Thus, the peptide increases renal vascular resistance, increases plasma renin activity, decreases urine flow, and decreases urinary sodium excretion (see Ref. 34 for review). It is of interest to note that we have recently reported that doses of endothelin lower than those used in the current study, doses that cause an increase in MAP but do not markedly affect the GFR, induce a pressure-dependent natriuresis and diuresis (35). Thus, the fact that lead attenuates the ET-induced increase in MAP but abolishes the decrease in the GFR appears to have exposed a pressure-dependent renal event. This interpretation is further supported by the nickel data which illustrate that at 4.8 nmoles/min $NiCl_2$, a dose which only affects ET-induced changes in GFR but not MAP, result in a sustained natriuretic and diuretic response to the peptide. It is also of interest to note that in most rats used in the current study, either control or those treated with lead or nickel, endothelin caused an increase in fractional excretion of sodium, a phenomenon which is consistent with the pressure-related inhibition of sodium reabsorption that occurs proximal to the thick ascending limb (35).

In summary, data from the current study illustrate that lead inhibits endothelin-induced increases in mean arterial blood pressure and decreases in the glomerular filtration rate and that a Ca^{2+}-related process may be involved in the systemic component. Nickel also inhibits both the systemic and renal actions of the peptide, but, compared with lead, the inhibition of the systemic component requires a higher dose.

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