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Neurohumoral Blood Pressure Regulation in Lead Exposure

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Previous human studies demonstrated that lead exposure may modify the metabolism of catecholamines and of hormones controlled by the hypothalamo-pituitary axis and may affect the kallikrein-kinin system. This paper reports unpublished data on the plasma renin activity of lead-exposed workers; these results are in agreement with those of previous human and experimental studies suggesting that the synthesis or release of renin is increased after short and moderate exposure to inorganic lead and reduced whenever the exposure is prolonged.

Previous experimental investigations demonstrated that lead may act on the cardiovascular system, with effects on the renin-angiotensin system, on the reactivity to stimulation of peripheral catecholaminergic receptors, on sympathetic and vagal tone, and on reactivity to the stimulation of baroreceptors. This paper reports the results of a study on male Sprague-Dawley rats that received 0, 15, 30, and 60 μg/mL of lead in drinking water for 18 months. Blood pressure was increased in the rats receiving 30 and 60 ppm of lead; cardiac inotropism was augmented only in those receiving the higher dose of the metal, and heart rate was not modified. Cardiovascular responses to agonists indicated that lead exposure affects the renin-angiotensin system and induces sympathetic hyperactivity by acting on central and peripheral sympathetic junctions increasing the responsiveness to stimulation of α_2-adrenoreceptors and by increasing the reactivity to stimulation of cardiac and vascular β-adrenergic and dopaminergic receptors. The cAMP-dependent availability of Ca^{2+} for contractile mechanisms of the cardiovascular muscle cells was affected by lead.

Human Studies

Several investigations on humans and experimental animals have attempted to explain the effects of lead exposure on several of the regulatory mechanisms for blood pressure (BP). In patients heavily intoxicated by lead (*1*), there were alterations of the hormones produced in the testis, adrenal cortex, and thyroid and alterations of those hormones regulated by the hypothalamo-pituitary axis. Lead-exposed children (*2*), but not hospitalized lead-exposed workers (*3*), had an increase of urinary catecholamines and their metabolites.

Urinary kallikrein (an enzyme synthesized in the kidney), which activates kinins (peptides with vasodilatory and natriuretic action), was reduced in patients with essential hypertension. Urinary kallikrein activity was normal in young, healthy lead-exposed men and was low or absent in old, hypertensive and/or nephropathic lead-exposed subjects (*4,5*). In 22 hospitalized lead-exposed workers, there was a weak but statistically significant correlation between urinary kallikrein activity and plasma renin activity (PRA) (*5*).

The present study describes all the cases of lead-exposed workers hospitalized at Catholic University of Rome, in which PRA was determined with previously described procedures (*5*). PRA was determined in 34 workers and in 21 control subjects both in the supine position and after 1 hr of programmed activity in the upright position. The workers were divided into four groups, on the basis of their age and length of exposure to inorganic or tetraethyl lead: group 1, 15 workers younger than 38 years with exposure to inorganic lead for less than 8 years; group 2, 4 workers older than 42 years with short exposure to inorganic lead; group 3, 8 workers older than 42 years and exposed for more than 12 years to inorganic lead; group 4, 7 men employed for more than 8 years in a factory producing tetraethyl lead.

In the subjects examined, 24-hr urine δ-aminolevulinic acid and lead excretion rates, before and after a 3-day versenate treatment, were determined; renal

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function was assessed by performing the ^{131}I-hippuran renogram and the creatinine clearance. Three of the 15 young lead-exposed men (group 1) presented arterial hypertension and another 2 had slight renal impairment; the mean PRA of this group, determined in the erect position, was significantly elevated in relation to that of control subjects of similar age (Table 1). The 4 workers in group 2 did not have modifications of PRA (there was only one case of a slight renal impairment). The patients in group 3 were suffering from arterial hypertension, nephropathy, or myocardial atherosclerosis. The PRA of this group manifested blunted or suppressed responses to stimulation by standing. Six of the 7 workers exposed to tetraethyl lead, with low lead body burden (group 4), presented hypertension or renal impairment, but PRA was reduced in only one case.

The workers with hypertension and/or nephropathy presented different levels of PRA in relation to the length of exposure to lead: in the 5 young workers from group 1, PRA was greatly augmented following the change of position; in the older workers of group 3, the response to the stimulation was reduced.

It seems likely that short lead exposure of healthy young subjects may increase the synthesis and/or release of renin from the juxtaglomerular cells; prolonged exposure may reduce the response of the juxtaglomerular apparatus to stimulations. A recent study of Campbell et al. (*6*) found increased PRA and plasma aldosterone in men with occupational exposure to lead for a relatively short period.

The results of the present study are also in agreement with the experimental investigations of Victery et al. (*8–10*), which demonstrated an increase in PRA in rats after a short period of lead exposure and reduced PRA whenever the exposure of the animals to the same doses of lead was initiated *in utero* and continued for several months.

Experimental Studies

In vivo and *in vitro* experimental studies have demonstrated that lead exposure may modify the mechanisms regulating the BP by acting not only on the juxtaglomerular apparatus (*8–10*), but also on peripheral vascular receptors (*11–13*), on the sympathetic nervous system (*11–12*), and on myocardial contractility (*11–14*).

In previous studies (*11,12*), our group examined the cardiovascular function of male Sprague-Dawley rats receiving 50 μg/mL of lead in drinking water for 160 to 180 days. Mean blood lead values of control and exposed animals were 13 and 38 μg/mL, respectively; kidney values were 0.3 and 3.2 μg/g (dry weight); and brain levels were 0.1 and 1.5 μg/g. Lead-exposed rats showed, in comparison to controls, a mean increase of systolic BP of 56 mm Hg and of diastolic BP of 41 mm Hg. In these animals the maximum rate of rise of blood pressure (dP/dt) was also significantly augmented; heart rate (HR) did not differ from that of the controls. In the exposed rats the responses to norepinephrine [acting principally on α_1 and α_2-adrenoreceptors (*15,16*)] were augmented; responses to the lower doses of epinephrine (which stimulates principally β_1 and β_2-adrenoreceptors, inducing hypotension and increasing inotropism) were more evident. Responses to the higher doses of epinephrine (acting on both α- and β-adrenoreceptors, increasing BP and dP/dt) were unmodified in relation to the controls. In the exposed rats the pressor responses to angiotensin II were increased; responses to angiotensin I, which is transformed to angiotensin II by angiotensin converting enzyme, were unchanged, suggesting a possible inhibition of this enzyme. In the exposed animals, bilateral carotid occlusion, which stimulates afferent baroreflex pathways, induced lower effects on BP and dP/dt than in the controls. In the lead-exposed rats, hexamethonium (a blocker of the sympathetic post-

Table 1. Urinary lead and plasma renin activity in groups of workers with different age and length of exposure to inorganic or tetraethyl lead[a]

No. of cases	Age, years	Exposure, years	Cases with hypertension or nephropathy	Urine lead, μg/24 hr		PRA, ng/mL-hr	
				Before EDTA	Max during EDTA[b]	Supine	Erect
Group 1: young workers with short exposure to inorganic lead							
15	28 ± 2	3 ± 1	5	101 ± 16	2092 ± 333	2.10 ± 0.43	6.36 ± 0.98*
Group 2: old workers with short exposure to inorganic lead							
4	46 ± 2	3 ± 1	1	57 ± 14	1537 ± 302	0.70 ± 0.28	2.05 ± 0.42
Group 3: old workers with prolonged exposure to inorganic lead							
8	53 ± 2	27 ± 3	7	134 ± 30	2805 ± 1513 (4)	0.87 ± 0.36	1.04 ± 0.34† (7)
Group 4: old workers with prolonged exposure to tetraethyl lead							
7	48 ± 4	18 ± 3	6	29 ± 5	657 ± 112	0.80 ± 0.19	2.37 ± 0.64
Young controls							
11	27 ± 2			< 50		0.90 ± 0.23	3.32 ± 0.47
Old controls							
10	52 ± 2			< 44		0.82 ± 0.34	2.52 ± 0.28

[a]All values represent means ± SEM. Numbers in parentheses are numbers of cases in which the determinations were made.
[b]1.5 g of $CaNa_2$-EDTA IV each day for 3 days.
*Significantly different from control group, $p < 0.05$.
†Significantly different from control group, $p < 0.01$.

ganglionic fibers, which induces systemic hypotension, bradycardia, and reduces dP/dt) presented greater effects than in the control animals. On the other hand, lead exposure reduced the effects of vagotomy. On the whole, this first experiment demonstrated that lead treatment in rats induces baroreflex hyposensitivity, peripheral sympathetic hyperactivity, hypersensitivity to the stimulation of both α- and β-adrenoreceptors, and reduction of vagal tone.

Recent Findings

The purpose of the present study was to further investigate the effects of chronic lead exposure in rats on the renin-angiotensin system, on the cardiovascular reactivity to catecholaminergic agonists, on some neurosynaptic events at the sympathetic junction, as well as on the availability of Ca^{2+} and cAMP in the cardiovascular muscle cell for contractile mechanisms.

Materials and Methods

Forty weanling male Sprague-Dawley rats, randomly divided in four equal groups and fed a standard laboratory diet, received in deionized drinking water 0, 15, 30, and 60 μg/mL of lead (as acetate) for 18 months. At the end of lead exposure, the rats were anesthetized with sodium thiopental (50 mg/kg IP). The trachea was cannulated to allow spontaneous breathing. The body temperature of the animals was kept constant at 37°C by an electrically heated table. For assessing cardiohemodynamic parameters, polyethylene catheters were placed in the left femoral artery for recording BP and in the right femoral vein for drug administration. Systolic and diastolic BP were measured by means of a P23Db Statham pressure transducer and averaged electronically. A Biotronex derivative computer was used for determining (by differentiating the pulsatile aortic BP) the dP/dt as index of cardiac inotropism. Heart rate was measured by a Beckman cardiotachometer coupler, which was triggered by the R-peak of the lead II electrocardiogram. The cardiovascular parameters were continuously monitored on a Beckman-type RM dynograph recorder.

Pressor, inotropic, and chronotropic responses were determined following IV injection of the following substances: phenylephrine (10 μg/kg, Sigma); isoprenaline (0.625 μg/kg, Merck); dopamine (12.5 μg/kg, Merck); IV infusion of clonidine (25 μg/kg, Boehringer Ingelheim); cocaine (2.5 mg/kg, Angelini); angiotensin I and II (0.50 μg/kg, Sigma) before and 30 min after IP administration of captopril (5 mg/kg; Squibb); papaverine (2 mg/kg, Merck); verapamil (125 μg/kg, Knoll AG); and dibutyryl adenosine 3′-5′-monophosphate (dBcAMP, 5 mg/kg; Fluka AG). All drugs were dissolved in 0.9% saline solution and were injected in a volume of 25 μL over a period of 5 sec (with the exception of clonidine administered by IV infusion over a 2-min period and captopril administered IP). The catheter was flushed with 50 μL of 0.9% saline solution. All doses of the administered substances were expressed in terms of free bases and peak responses were considered.

Blood samples were collected for the determination of lead. The animals were killed by exsanguination and organs were excised for determining the lead content (referred to as the wet weight) and for histological examination. Lead content of blood and organs was determined by atomic absorption spectrophotometry (*16*).

The statistical comparison of the data was made by Dunnett's *t*-test for multiple comparison.

Results

Throughout the exposure period, body weight and general appearance of the animals were not modified by the lead exposure. Lead was increased in blood, kidney, and brain of the treated rats at levels related to the administered dose of the metal (Table 2).

The rats receiving 15 ppm of lead did not demonstrate any modification of the basal cardiovascular parameters (Fig. 1). Diastolic BP was significantly augmented in the rats receiving 30 ppm of lead; the animals treated with 60 ppm of lead presented increases of both systolic and diastolic BP, of the dP/dt, but not of the heart rate.

In the rats receiving 15 ppm lead, only the pressor responses to angiotensin I and to dibutyryl-cAMP were modified (Table 3). All the exposed rats showed similar, reduced BP responses to angiotensin I, but not to angiotensin II. Captopril, an inhibitor of the angiotensin I converting enzyme, produced similar decreases in both control and exposed animals (about 20 mm Hg); however, captopril did not modify the responses to angiotensin I and II in the rats receiving 15 ppm of lead, although it reduced the pressor responses to the same agonists in the animals treated with the higher doses of lead. In all the exposed rats, dibutyryl-cAMP, which enters cardiac and vascular muscle cells, induced a hypotensive response. Dibutyryl-cAMP provoked an increase of BP in the controls. In the rats exposed to 30 and 60 ppm of lead, the hypotensive responses to isoprenaline, a $\beta_{1\text{-}2}$-adrenoceptor agonist, were potentiated, and the moderate hypertensive responses to dopamine were reversed. In

Table 2. Lead in blood, kidney and brain of rats receiving different doses of lead in drinking water for 18 months.[a]

Group	Dose, μg/mL	Blood, μg/dL	Kidney μg/g[b]	Brain, μg/g[b]
Control	0	3.9 ± 0.2	0.08 ± 0.02	< 0.03
Exposed	15	7.4 ± 0.5*	0.4 ± 0.1*	< 0.07
Exposed	30	11.5 ± 0.9*	1.0 ± 0.1*	< 0.08
Exposed	60	16.7 ± 0.6*	2.7 ± 0.6*	0.8 ± 0.1*

[a]The values represent means ± SEM.
[b]Lead contents of kidney and brain are related to the wet weight.
*Significantly different from control group, $p < 0.05$.

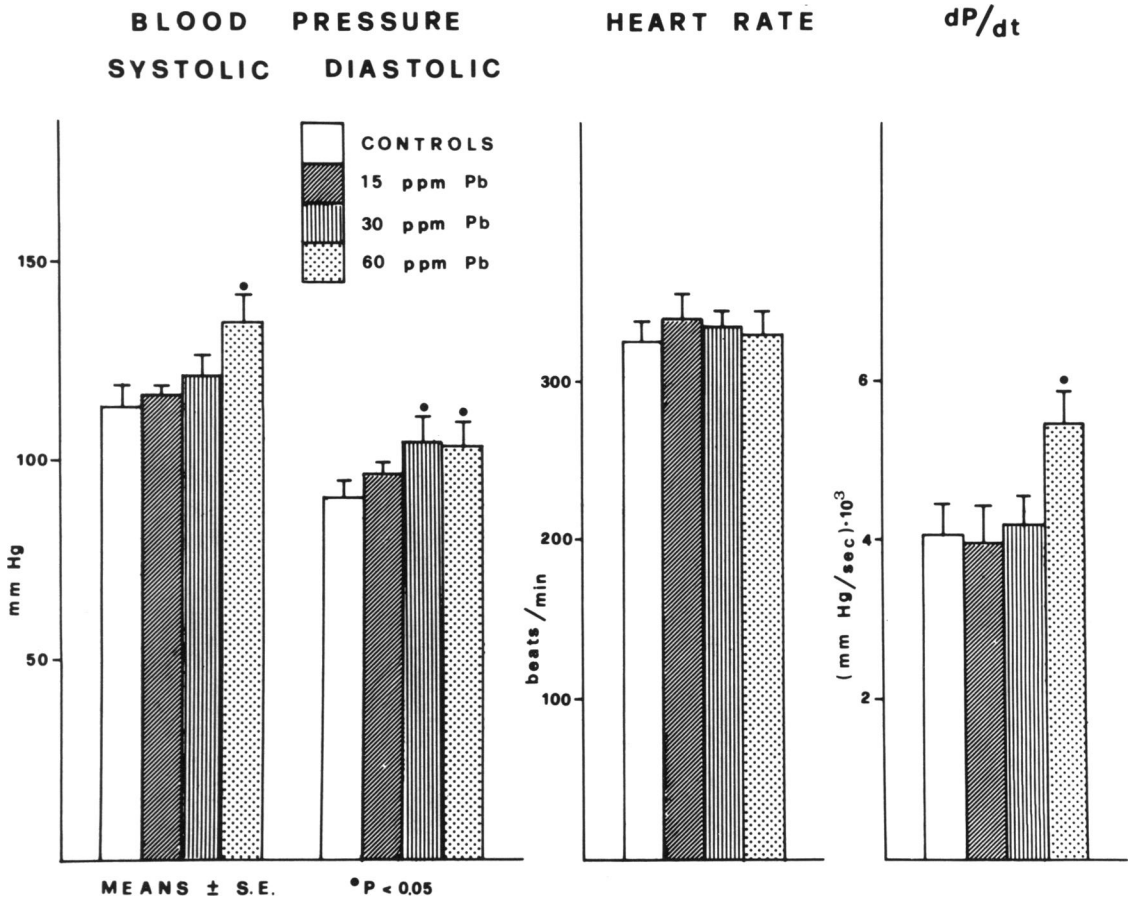

FIGURE 1. Systolic and diastolic blood pressure, heart rate, and maximum rate of rise of the left ventricular pressure (dP/dt) in rats receiving 0, 15, 30, and 60 ppm of lead in drinking water for 18 months.

Table 3. Peak changes in mean blood pressure following IV administration of various substances in rats receiving different doses of lead in drinking water for 18 months.

	Changes in mean blood pressure, mm Hg			
		Exposure, μg/mL of lead		
Substance administered	Control	15	30	60
Phenylephrine, 10 μg/kg	+ 39 ± 2	+ 40 ± 2	+36 ± 5	+ 35 ± 2
Isoprenaline, 0652 μg/kg	− 22 ± 3	− 28 ± 3	− 34 ± 3*	− 41 ± 4*
Dopamine, 12.5 μg/kg	+ 13 ± 2	+ 12 ± 2	− 2 ± 1*	− 13 ± 2*
Tyramine, 450 μg/kg	+ 46 ± 4	+ 38 ± 3	+ 36 ± 4	+ 27 ± 6*
Clonidine, 25 μg/kg	− 11 ± 3	− 13 ± 5	− 17 ± 5	− 25 ± 1*
Cocaine, 2.5 μg/kg	+ 19 ± 3	+ 22 ± 3	+ 18 ± 4	+ 16 ± 2
Angiotensin I, 0.5 μg/kg				
Before captopril[a]	+ 42 ± 2	+ 26 ± 2*	+ 24 ± 3*	+ 32 ± 2*
After captopril	+ 43 ± 3	+ 24 ± 3	+ 15 ± 3*	+ 10 ± 3*
Angiotensin II, 0.5 μg/kg				
Before captopril	+ 47 ± 7	+ 49 ± 7	+ 50 ± 4	+ 42 ± 3
After captopril	+ 52 ± 4	+ 51 ± 8	+ 44 ± 5	+ 29 ± 5*
Papaverine, 2 mg/kg	− 19 ± 3	17 ± 2	− 18 ± 2	− 23 ± 3
Verapamil, 125 μg/kg	− 27 ± 5	− 29 ± 2	− 31 ± 3	− 26 ± 5
Dibutyryl cAMP, 5 mg/kg	+ 56 ± 5	− 12 ± 3*	− 15 ± 4*	− 13 ± 4*

[a]Captopril (5 mg/kg) was administered by IP route. It decreased blood pressure similarly in control and lead-exposed groups (~ 20 mm Hg).
*Significantly different from control group, $p < 0.05$.

the rats receiving 60 ppm lead, the hypertensive effects induced by tyramine (releasing the labile pool of norepinephrine from the axonal postganglionic adrenergic terminals) were reduced; the hypotensive effects of clonidine (which reduces the sympathetic tone, principally by stimulating α_2-adrenoreceptors of central sympathetic junctions) were potentiated. On the other hand, the cardiovascular responses to each of the following pharmacologic agents were not modified by lead exposure: phenylephrine (a specific α_1-adrenoreceptor agonist); papaverine (a smooth muscle relaxant that inhibits the phosphodiesterase); cocaine (a blocker of the neuronal uptake of noradrenaline); and verapamil (a calcium antagonist that inhibits both in myocardial and vascular muscle cells the slow influx of calcium through receptor operated channels). In the exposed rats, heart rate was not modified by these substances. Histological examination by light microscopy did not reveal alterations in the organs of the exposed rats.

Discussion

This study confims that long-term exposure of rats to lead is able to increase both blood pressure and cardiac inotropism without morphological modifications, as observed by light microscopy.

The reduced responses to angiotensin I, but not to angiotensin II, in the lead-exposed rats may be explained by a possible inhibition of the converting enzyme and/or by an increase in plasma renin activity augmenting the level of angiotensin I in the plasma. The reduced responses to angiotensin I and II after captopril in the rats receiving higher doses of lead may be related to an effect of the metal on the mechanisms involved in the homeostatic regulation of the renin-angiotensin system.

The increased hypotensive response to clonidine, resulting from stimulation of the α_2-adrenoreceptors (*13*), also with inhibition of the release of norepinephrine from the central sympathetic axonal endings, indicates that the increase of the sympathetic tone, demonstrated in the first experiment by the augmented responses to the ganglioplegic hexamethonium (*11,12*), was centrally mediated. Moreover, the reduction observed in the treated animals of the tyramine-sensitive pool of norepinephrine in the sympathetic endings may be related to increased activity of the postganglionic fibers.

In the lead-exposed rats the unchanged cardiovascular effects following phenylephrine or cocaine prove that lead neither significantly affects the reactivity to the stimulation of α_1-adrenoreceptors nor the neuronal uptake of noradrenaline.

The cardiovascular responses induced by the activation of β-adrenoreceptors by isoprenaline and of dopaminergic receptors by dopamine were augmented in lead-exposed rats. It is important to point out that α_2-, β_1-, and β_2-adrenoreceptors, as well as the dopaminergic receptors, act together to regulate the cyclic nucleotide-dependent availability of calcium for contractile systems (*15,16*). Dibutyryl cAMP, which enters cardiovascular muscle cells, induced reverse effects in the lead-exposed rats. This may be due to a depletion of cAMP produced from ATP due to impairment of the high energy phosphate pathway (*14*). On the other hand, it must be pointed out that the altered cAMP-dependent availability of Ca^{2+} for contractile mechanisms in cardiovascular muscle cells was related neither to the phosphodiesterase activity nor to the verapamil-sensitive Ca^{2+} channels.

This study confirms that chronic lead exposure affects the cardiovascular system, not only by inducing metabolic changes (as those related to the metabolism of calcium or of the high energy phosphate pathway) (*7,14,16*) which are able to produce neurohumoral disorders, but also by inducing neurogenic and humoral effects that may involve the regulation of metabolic processes.

REFERENCES

1. Cullen, M. R., Kayne, R. D., and Robins, J. M. Endocrine and reproductive dysfunction in men associated with inorganic lead intoxication. Arch. Environ. Health 39: 431–440 (1984).
2. Silbergeld, E., and Chisolm, J. J. Lead poisoning. Altered urinary catecholamine metabolites as indicators of intoxication in mice and children. Science 192: 153–155 (1976).
3. Boscolo, P., Carelli, G., Liberale, I., Lombardo, P., Menini, E., and Sciamanna, V. Urinary catecholamines and vanillylmandelic acid of lead-exposed workers: Effect of EDTA and zinc treatment. Acta. Med. Rom. 20: 121–126 (1982).
4. Boscolo, P., Porcelli, G., Cecchetti, G., Salimei, E., and Iannaccone, A. Urinary kallikrein activity of workers exposed to lead. Br. J. Ind. Med. 35: 226–229 (1978).
5. Boscolo, P., Galli, G., Iannaccone, A., Marino, F., Porcelli, G., and Troncone, L. Plasma renin activity and urinary kallikrein excretion in lead-exposed workers as related to hypertension and nephropathy. Life Sci. 28: 175–184 (1981).
6. Campbell, B. C., Meredith, P. A., and Scott, J. J. C. Lead exposure and changes in the renin-angiotensin-aldosterone system in man. Toxicol. Lett. 25: 25–32 (1985).
7. Sharp, D. S., Becker, C. E., and Smith, A. H. Chronic low-level lead exposure. Its role in the pathogenesis of hypertension. Med. Toxicol. 2: 210–232 (1987).
8. Victery, W., Vander, A. J., Shulak, J. M., Schoeps, P., and Julius, S. Lead, hypertension and the renin-angiotensin system in rats. J. Lab. Clin. Med. 99: 354–362 (1982).
9. Victery, W., Vander, A. J., Markel, H., Katzma, L., Shulak, J. M., and Germain, C. Lead exposure, begun *in utero*, decreases renin and angiotensin II in adult rats. Proc. Soc. Exp. Biol. Med. 170: 63–67 (1982).
10. Victery, W., Vander, A. J., Schoeps, P., and Germain, C. Plasma renin is increased in young rats exposed to lead *in utero* and during nursing. Proc. Soc. Exp. Biol. Med. 172: 1–7 (1983).
11. Iannaccone, A., Carmignani, M., and Boscolo, P. Neurogenic and humoral mechanisms in arterial hypertension of chronically lead-exposed rats. Med. Lav. 72: 13–21 (1981).
12. Carmignani, M., Boscolo, P., Ripanti, G., and Finelli, V. N. Effects of chronic exposure to cadmium and/or lead on some neurohumoral mechanisms regulating cardiovascular function in the rat. In: International Conference on Heavy Metals in the Environment. Vol. 1, Heidelberg, September 1983 (G. Müller, Ed.), CEP Consultants Ltd., Edinburgh, 1983, pp. 513–516.
13. Webb, R. C., Winquist, R. J., Victery, W., and Vander, A. J. *In vivo* and *in vitro* effects of lead on vascular reactivity in rats. Am. J. Physiol. 241: H211–H216 (1981).

14. Kopp, S. J., Barany, M., Erlanger, M., and Perry, E. M. The influence of chronic low level cadmium and/or lead on myocardial contractility related to phosphorylation of cardiac myofibrillar proteins. Toxicol. Appl. Pharmacol. 54: 48–56 (1980).
15. Namm, D. H. The role of cyclic nucleotides in vasculature. In: Cyclic Nucleotides II. Physiology and Pharmacology (J. W. Kebabian and A. Nathanson, Eds.), Handbook of Experimental Pharmacology, Vol. 58/II. Spring-Verlag, Berlin, 1982, pp. 683–690.
16. Churchill, P. C. Second messengers in renin secretion. Am. J. Physiol. 249: F175–F184 (1985).
17. Selander, S., and Cramér, K. Determination of lead in blood by atomic spectrophotometry. Br. J. Ind. Med. 25: 209–213 (1968).