

Effects of low level of lead exposure on responsiveness of the rat isolated heart to adrenergics

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ABSTRACT

Background: There are controversial reports about the exact mechanisms of lead-induced hypertension, but many factors such as alteration in the responsiveness of cardiovascular system to endogenous substances including catecholamines could be one of the mechanisms involved. In present study, the effect of exposure to 100 ppm lead acetate by drinking water (in the periods of 4, 8 and 12 weeks) on the responsiveness of rat isolated beating heart to β -adrenergics was investigated, using Langendorff isolated heart setup.

Methods: The isolated hearts were perfused with Krebs-Henseleit solution at 37°C and pH=7.4 and gassed with 95% O₂ + 5% CO₂. The rate (chronotropic) and contractile (inotropic) responses of the heart to β -adrenergics (isoproterenol and dobutamine) were recorded by adding these agents at multiple concentrations to the perfusion solution.

Results: The blood pressure in 8- and 12-week lead-treated groups was significantly increased compared with those of the control group (P<0.01). The chronotropic response to many doses of isoproterenol (as $\beta_{1,2}$ -adrenergic) in only 12-, but not in 4- and 8-week lead-treated groups was significantly increased, as compared with those of control (P<0.05). The inotropic response to this drug was also significantly increased in both 8- and 12-week lead-treated rats (P<0.05, P<0.01). Similar findings were observed in the dobutamine (as selective β_1 -adrenergic) treated groups, but the contractile response of the latter agent was greater than the isoproterenol.

Conclusions: Low-level of lead increases blood pressure and both chronotropic and inotropic effects of β -adrenergics. These effects could imply an important role in the pathogenesis of lead-induced hypertension.

Key words: Adrenergic system, Contractility, Hypertension, Lead acetate

INTRODUCTION

Lead is one of the important heavy metals that is distributed widely in our environment as a consequence of progressive use in industries (1). For this reason, it became an important environmental pollutant that exerts toxic effects on human health (2). Lead intoxication may cause neurological, hematological, gastrointestinal and cardiovascular dysfunctions in human and

experimental animals (3,4,5). Several epidemiological and experimental reports have documented that lead is a factor of cardiovascular impairment (6,7). In recent decades, the relationship between levels of lead in blood and blood pressure has extensively been investigated. In this regard, chronic exposure to low levels of lead has been shown to cause hypertension in both human and animals (8, 9,10). Many

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mechanisms are proposed for lead-induced hypertension, including alteration in calcium (Ca^{+2}) flux, lowering the Ca^{+2} binding capacity in intracellular Ca^{+2} stores, leading to an increase intracellular Ca^{+2} concentration (11,12), inhibition of sodium pump (13), increased activity of renin-angiotensin system (14), altered kallikrein-kinin system causing decreased plasma levels of bradikinin (9,15), and increased cardiovascular sensitivity to endogenous substances such as catecholamines (10). However, there is not much data on cardiovascular effects of either low or moderate levels of lead exposure and even some controversies exist in this regard (15,16). The aim of this study was to determine the subchronic effects of low-level (100 ppm) of lead acetate on blood pressure and responsiveness of the isolated heart to β -adrenergics (isoproterenol and dobutamine) in male rat.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Pasteur Institute, Tehran-Iran) of 250-300 g body weight were used. They had free access to food and water while kept in animal house at a temperature of $23\pm 2^\circ\text{C}$ with a 12-h light/dark cycle. Animals were randomly divided into control and three lead-treated (4-, 8- and 12-weeks) groups. Six animals were allocated to each group.

Exposure protocol: Three lead-treated groups were given 100 ppm (0.01%) lead acetate in their drinking water for periods of 4, 8 and 12 weeks but, control rats were given drinking water without lead acetate.

Chemicals: Isoproterenol (as $\beta_{1,2}$ -drenoceptor agonist) and its antagonist, propranolol and, dobutamine (as β_1 -adrenoceptor agonist) were obtained from Sigma co, USA. Atenolol (as a β_1 -antagonist) was a generous gift from Temad D.P.P.C co, Iran. All other chemicals such as lead acetate and all compounds of Krebs-Henseleit solution were obtained from Merck co, Germany.

Blood pressure measurement: At the end of intoxication periods, the animals were anesthetized with a mixture of ketamin (75 mg/kg) and xylazine (25 mg/kg). Then, the

rat tail was placed inside the tail cuff and the cuff was inflated and released a few times to allow the animal to be conditioned to the procedure. Systolic blood pressure values (three consecutive readings) were recorded by a tail sphygmomanometer (PE 300, Narco Bio-systems, USA) attached to a polygraph (MK III-P, Narco Bio-systems) and averaged for analysis.

Determination of lead in blood: Lead content of whole blood was measured using an atomic absorption spectrophotometer (shimadzu 680A, with graphite furnace, shimadzu, Japan) and expressed as micrograms per deciliter ($\mu\text{g}/\text{dl}$) (17).

Surgical procedure: The animals were anesthetized with a mixture of ketamin (75 mg/kg) and xylazine (25 mg/kg) after intraperitoneal injection of 500 IU heparin as anticoagulant. Thereafter, the animal thorax was opened from bilateral axillary's lines up to first rib, under artificial ventilation. Then, the ascending aorta was cannulated and the exposed heart was carefully isolated from the body (all vessels were cut out) and transferred immediately (among maximum 30 seconds) to Langendorff rat isolated heart set up. The isolated hearts were continuously perfused with Krebs-Henseleit solution (at 37°C , $\text{pH}=7.4$ and gassed with 95% O_2 + 5% CO_2) with compositions of (in mM/l): NaCl, 118; KCl, 7.4; NaHCO_3 , 25.0; KH_2PO_4 , 1.2; CaCl_2 , 2.5; $\text{MgSO}_4.7\text{H}_2\text{O}$, 1.2; and Glucose, 11.0. A 15-min period was allowed in order for the heart to reach a steady state condition prior to any treatment (18). For measuring the effects of drugs on heart, each drug in different concentrations was added to the solution perfusing the heart, and its effects were recorded.

Parameters recorded:

ECG: The HR was calculated from R-R intervals in ECG. The ECG was recorded by 3 surface silver electrodes (2 active and 1 reference) placed on the surface of isolated heart in the axis of lead II with a Hi-gain coupler (Narco Bio-system, USA). R-R intervals and arrhythmogenicity were processed.

Cardiac contractility: A strain-gauge was connected to the apex of the heart and the

contractile signals were sent to an isotonic myograph transducer via an isotonic myograph detector (Narco Bio-system, USA) attached to the strain gauge.

Finally, all online data taken from the heart were analyzed using Long soft (Iran, version 1.1) program.

Statistical analysis:

All results were expressed as the mean \pm SEM and statistical differences were evaluated by unpaired *t*-test (only for blood pressure data of control and three lead treated groups) and *two ways ANOVA* followed by *Tukey HSD* post hoc test (for other parameters). *P* values less than 0.05 was considered significant.

RESULTS

There was no significant difference in body weight between control and all lead-treated groups before and after treatment periods. The blood lead concentration of treated rats after 12 weeks was significantly higher than controls (26.84 \pm 2.23 μ g/dl versus <2 μ g/dl).

Blood pressure. Systolic BP was increased by lengthening exposure periods from 0 to 12 weeks (100.68 \pm 1.01 vs. 129.18 \pm 2.64 mmHg) (figure 1). The increment of BP in 4-week lead-treated groups was not significant, but in 8- and 12-week lead-treated rats was significant, as compared with those of respective controls. This parameter was unchanged in control groups throughout 12 weeks of the experiment.

Chronotropic effects of drugs:

Chronotropic (HR) responses to different doses of isoproterenol (figure 2) and dobutamine (figure 3) in 4- and 8-week lead-treated and control groups were not significantly different (except maximum dose of isoproterenol, 5 \times 10⁻⁶M, *P*<0.05) and the respective dose-response curves overlap. However, the dose-response curves for both drugs in 12-week lead-treated groups were significantly shifted to the left side and upward (*P*<0.05). Thus, short term (4-8 weeks) exposure to lead could not affect on HR response, while in the late phases of exposure (12 weeks), this response in lead-treated rats increased significantly compare to control.

Inotropic effects of drugs:

The contractile (inotropic) responses of isolated heart to isoproterenol are shown in figure 3. There were no significant differences between control and 4-week lead-treated groups. It seems that acute lead exposure could not change either the contractility or sensitivity of the isolated heart to β -adrenergics. While, contractile responses of isoproterenol in 8- and 12-week lead-treated groups were significantly increased (*P*<0.05, *P*<0.01) (figure 4).

Similar findings were obtained with dobutamine (figure 5). Dobutamine contractile response curves in 8- and 12-weeks lead-treated groups were significantly higher than control group (*P*<0.05, *P*<0.01). As it has been shown, rise in contractility is steeper in the response to dobutamine compare to isoproterenol. Moreover shifting contractile curves to upward and left side shows the augmentation of the β -adrenoceptors sensitivity by lead.

The chronotropic and inotropic responses of isoproterenol and dobutamine were examined in the presence of Propranolol (as general $\beta_{1,2}$ -antagonist, 10⁻⁶ M) and atenolol (as selective β_1 -antagonist, 10⁻⁵ M). These antagonists reduced the responses to general and selective agonists, suggesting that observed responses are produced by stimulation of corresponding receptors (Table 1 and 2).

Finally, in 42% of 12-week lead-treated rats the signs of arrhythmias have been seen following administration of adrenergic drugs to the heart. These signs were not seen in any other groups.

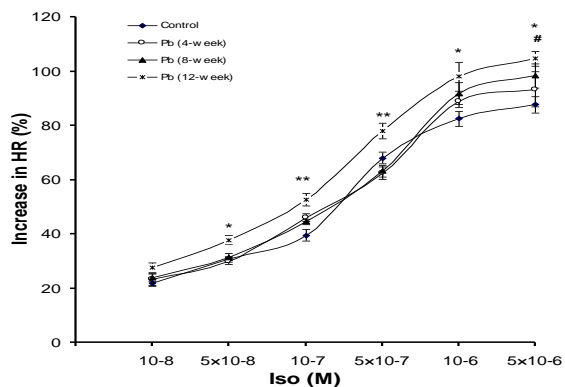


Figure 1. The effect of isoproterenol (Iso, M) on HR of control and lead-treated (Pb) rats (Data presented as mean \pm SEM, n=6; #,*P<0.05, **P<0.01).

(In all graphs, #: Differences between control and 8-week lead-treated rats, *: Differences between control and 12-week lead-treated rats.)

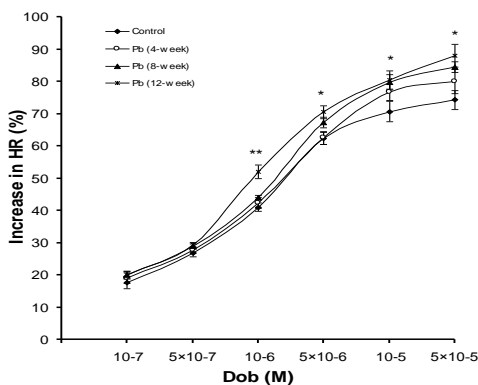


Figure 2. The effect of Dobutamine (Dob, M) on HR of control and lead-treated (Pb) rats (Data presented as mean \pm SEM, n=6; *P<0.05).

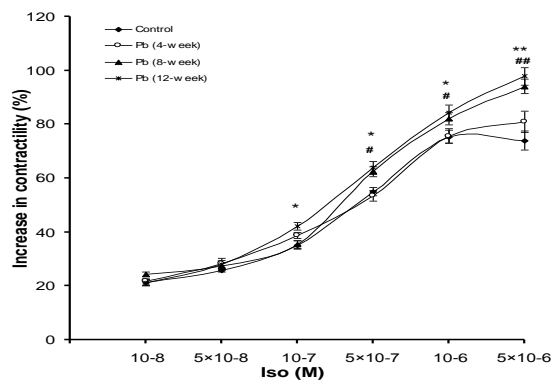


Figure 3. The effect of isoproterenol (Iso, M) on cardiac contractility of control and lead-treated (Pb) rats (Data presented as mean \pm SEM, n=6; #,*P<0.05; ##, **P<0.01).

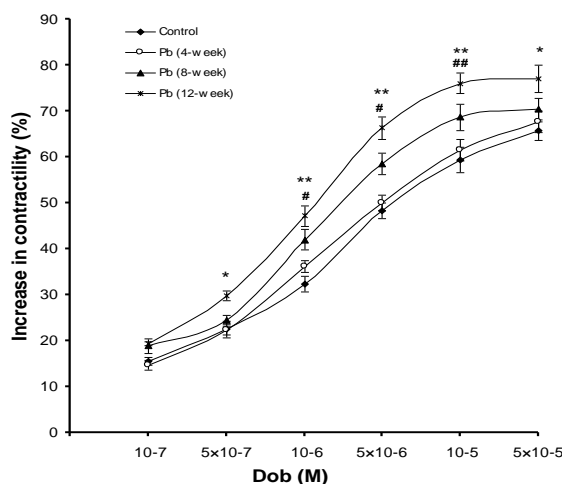


Figure 4. The effect of Dobutamine (Dob, M) on cardiac contractility of control and lead-treated (Pb) rats (Data presented as mean \pm SEM, n=6; #,*P<0.05; ##, **P<0.01).

Table 1. Maximum chronotropic responses (CR_{max}) and inotropic responses (IR_{max}) induced by isoproterenol and dobutamine and inhibition of these responses by their relative antagonist (propranolol and atenolol, respectively).

Agents (max. dose)	(% CR _{max} in groups)				(% IR _{max} in groups)			
	Control	4-week Pb	8-week Pb	12-week Pb	Control	4-week Pb	8-week Pb	12-week Pb
Iso (5x10 ⁻⁶ M)	87.67 \pm 3.06	93.33 \pm 6.51	98.33 \pm 4.4 [#]	104.67 \pm 3.67 [*]	74 \pm 3.66	81 \pm 3.93	94 \pm 2.7 ^{##}	97.83 \pm 3.22 ^{**}
Iso (5x10 ⁻⁶ M) and Prop (10 ⁻⁶ M)	36.94 \pm 2.11	42.06 \pm 1.6	41.09 \pm 1.97	47.97 \pm 2.09	38.7 \pm 1.61	40 \pm 1.81	43.6 \pm 1.77	47.4 \pm 2.24
Dob (5x10 ⁻⁵ M)	74.33 \pm 2.92	80 \pm 3.68	84.5 \pm 2.57	88 \pm 3.47 [*]	65.67 \pm 2.02	67.67 \pm 2.33	70.5 \pm 2.33	77 \pm 2.94 [*]
Dob (5x10 ⁻⁵ M) and Ate (10 ⁻⁵ M)	30.62 \pm 1.69	33.1 \pm 1.81	33.42 \pm 1.77	37.3 \pm 2.24	26.9 \pm 0.6	27.7 \pm 1.42	28.3 \pm 1.6	31.7 \pm 1

Iso: Isoproterenol, Dob: Dobutamine, Prop: Propranolol, Ate: Atenolol. Values are presented as mean \pm SEM, n=6 in all groups; #,*p<0.05 and ##,**P<0.01 in lead-treated (Pb) groups as compared with values of respective control groups..

DISCUSSION

Alteration of cardiovascular responsiveness to β -adrenergics has been demonstrated in several models of hypertension. In previous studies, it has been explained that increased in vascular contractile responsiveness to adrenergic agonists is shown in lead-induced hypertension (10,14). However, there are controversial results among studies (19,20). In present study, this hypothesis which lead-induced hypertension could increase cardiac reactivity to β -adrenergics was investigated. As our findings demonstrated, lead could increase the responsiveness of isolated heart to β -adrenergics. Dose-response curves for the increased HR and cardiac contractility induced by isoproterenol or dobutamine were obtained in isolated hearts from control and 4-, 8- and 12-week lead-treated rats. From the differences in responses of isolated heart in lead-treated rats as compared to control animals to catecholamines, it can be concluded that the chronotropic and inotropic effects of both agents (isoproterenol and dobutamine) are significantly increased in 8-week and, especially 12-week lead-treated groups and the respective dose-responses curves shift to the left in these groups. Exposure to low level of lead after 4 weeks did not alter the responsiveness of isolated heart to catecholamines. However, arrhythmias followed by use of adrenergic drugs were also seen after 12 weeks lead exposure.

Increased chronotropic (19,21) and inotropic (19) effects of isoproterenol that augment β -adrenoceptors activity in the heart, were noted in some previous studies. However, a diminished chronotropic response to isoproterenol is also reported in a lead-poisoned patient (20), as well as a decreased inotropic effect of this drug is explained in the rat isolated hearts perfused with a solution containing lead acetate (22), which disagree with our results. The lead dosage used by these investigators was much greater than the dosage used in our experiment. So, this factor together with disparities in the experimental protocol may explain the differences observed. However,

increased contractile responses of adrenergics in blood vessels were noted in approximately all studies in this regard (10,23).

Increased cardiac reactivity to β -adrenergics and increased β -receptor activity could be due to alteration by lead in pre- and post-receptor events, such as an increase in the density of β -adrenoceptors in heart, a change in the structure of receptor leading to increase of the affinity of agonist to receptors or an increase in the activity of adenylate cyclase and as a result, an increase in the intracellular concentration of cAMP and Ca^{2+} . However, Chang and his colleagues (24) reported that lead exposure results in a reduction of cardiovascular β -adrenoceptor density and a diminution of cAMP accumulation in brain (25). While, in present study, the dose-response curves of lead-treated rats were shifted to upward and left side. This demonstrates that the sensitivity of β -adrenoceptors is augmented by lead. Increased activity of β -adrenoceptors can augments cardiac contractile force by intracellular Ca^{2+} accumulation. Moreover, as we showed, the difference between contractile responses of control and lead-treated groups elicited by dobutamine was greater than those of isoproterenol (the slope of these responses was steeper in the presence of dobutamine.). This permeably demonstrates that lead acts preferably through β_1 -adrenoceptors activation.

Furthermore, it has be found that chronic lead exposure in rats induces a higher cAMP-related availability of Ca^{2+} for contractile processes in both vascular and cardiac myocytes (19,26,27). In addition, in cardiomyocytes, the β_1 -adrenoceptor induced-increase of cAMP levels stimulates contractility by a prolonged influx of Ca^{2+} through the voltage gated Ca^{2+} channels (19). So, although the amounts of cAMP are likely reduced in the presence of lead (25), but the β_1 -adrenoceptor activation (sensitization) by lead will be able to increase cAMP even more than in the absence of lead exposure, despite a likely lower β_1 -adrenoceptor density (24).

In conclusion, the present findings demonstrate that exposure to low level of lead can increase chronotropic and inotropic effects of β -adrenergics. These responses may be mediated mostly by β_1 -adrenoceptors activation. Pathogenesis of lead-induced hypertension could be explained by increase in rate and contractility of the heart.

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