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Cardiac responsiveness to beta-adrenergics in rats with lead-induced hypertension

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Abstract
There are controversial reports about the exact mechanisms of lead-induced hypertension, but alteration in the responsiveness of cardiovascular system to catecholamines may be involved. In the present study, the effect of exposure to low level of lead acetate on responsiveness of isolated beating heart to β-adrenergics in male rats was investigated, using Langendorff isolated heart. Animals were randomly divided into 4 groups: control and 4, 8 and 12-week lead-treated groups. Lead-treated groups received 100ppm lead acetate in drinking water. The isolated hearts were perfused with Krebs-Henseleit solution at 37˚C and pH=7.4 under constant pressure and gassed with 95%O_2+5%CO_2. The blood pressure of anesthetized animals and chronotropic and inotropic responses of the isolated hearts to β-adrenergics (isoproterenol and dobutamine) were recorded separately and corresponding dose-response curves were obtained. 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 isoproterenol in only 12-week lead-treated group was significantly increased. The inotropic response to this drug was also significantly increased in both 8-and 12-week lead-treated rats ($P<0.05$). Similar findings were observed with dobutamine, but the contractile response of the latter agent was greater than the isoproterenol. Our results indicate that 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.

Keywords: Lead acetate, hypertension, contractility, adrenergic system.

Introduction

Lead is one of the important heavy metals, and due to widespread use in industry, it has became an important environmental pollutant that exerts toxic effects on human health (Jubery *et al*., 1997; Kimberlie and Charles, 1997). Lead intoxication may cause neurological, hematological, gastrointestinal and cardiovascular dysfunctions in human and experimental animals (Johnson, 1998; Lai *et al*., 2002; Badalzadeh *et al*., 2008). Based on epidemiological and experimental reports, lead is a factor of cardiovascular impairment (Pirkle *et al*., 1985; Lal *et al*., 1991). During the past decades, the relationship between levels of lead in blood and blood pressure has been investigated. In this field, chronic exposure to low levels of lead has been shown to cause hypertension in both humans and animals (Perry *et al*., 1988; Karimi *et al*., 2002; Heydari *et al*., 2006). Many 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 (Schanne *et al*., 1997; Goldstein, 1993), inhibition of sodium pump (Weiler *et al*., 1988), increased activity of renin-angiotensin system (Sharifi *et al*., 2004), altered kallikrein-kinin system causing decreased plasma levels of bradikinin (Karimi *et al*., 2002; Carmignani *et al*., 1999), and increased cardiovascular sensitivity to endogenous substances such as catecholamines (Heydari *et al*., 2006). Pathogenesis of lead-induced hypertension could be explained by increase in rate and contractility of the heart. However, there is not much data on cardiovascular effects of either low or moderate amounts of lead exposure and even some controversies exist in this regard (Carmignani *et al*., 1999; Evis *et al*., 1985). Therefore, the aim of this study was to determine the subchronic effects of low-level (100 ppm) of lead acetate on systolic blood pressure and responsiveness of the isolated heart to β-adrenergics (isoproterenol and dobutamine) in male rat.

Materials and Methods

Animals: In present experiment, male Sprague-Dawley rats 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±2˚C with a 12-h light/dark cycle. Animals were randomly divided into control and three lead-treated (4-, 8- and 12-weeks) groups. Twelve animals were allocated to each group which six animals were used for investigating the cardiac effects of isoprotrenol and other six animals for dobutamine.

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 received drinking water without any lead acetate.

Chemicals: Isoproterenol (as $\beta_{1,2}$-adrenoceptor agonist) and its antagonist, propranolol and, dobutamine (as selective β_1-adrenoceptor agonist) were obtained from Sigma co, USA. Atenolol (as a selective β_1-antagonist) was a generous gift from Temad D.P.P.C co, Germany. 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 exposure 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 (BP) 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 (µg/dl) (Abdollahi *et al*., 2000).

Surgical procedure: The animals were anesthetized 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 to a constant pressure Langendorff rat isolated heart set up. The isolated spontaneously beating hearts were continuously perfused with Krebs-Henseleit solution (at 37˚C, pH=7.4, gassed with 95%O_2 + 5%CO_2 and perfusion pressure of 70 mmHg) with compositions of (in mM/l): NaCl, 118; KCl, 7.4; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $MgSO_4.7H_2O$, 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 (Sutheland and Hears, 2000). For measuring the effects of drugs on heart, each drug in different concentrations was added to the solution perfusing the heart, and its effect was recorded in the period of 2min at steady state.

Parameters recorded

HR: The HR was calculated from R-R intervals in Electrocardiogram (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).

Cardiac contractile force: A strain-gauge was connected to the apex of the heart and the contractile signals were send to an isotonic myograph transducer via an isotonic myograph detector (Narco Bio-system, USA) attached to the strain gauge. In this situation, a 3-5g diastolic muscle stretch was placed on hearts relative to theirs size. Finally, all online data taken from the heart were analyzed using a homemade software (Long soft, version 1.1, Iran) program.

Statistical analysis

All results were expressed as the Mean ± SEM and statistical differences were evaluated by unpaired *t*-test (only for blood pressure data) 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 (23.54±2.06 µg/dl versus 1.97±0.43 µg/dl).

Blood pressure: Systolic blood pressure was increased by lengthening exposure periods from 0 to 12 weeks. Since BP values were similar to what has been reported previously (Heydari *et al*., 2006), these data were omitted from this study.

Chronotropic effects of drugs

Chronotropic (HR) responses to isoproterenol (figure 1) and dobutamine (figure 2) in 4- and 8-week lead-treated groups were not significantly different from control group (except maximum dose of isoproterenol, 5 µM, 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.

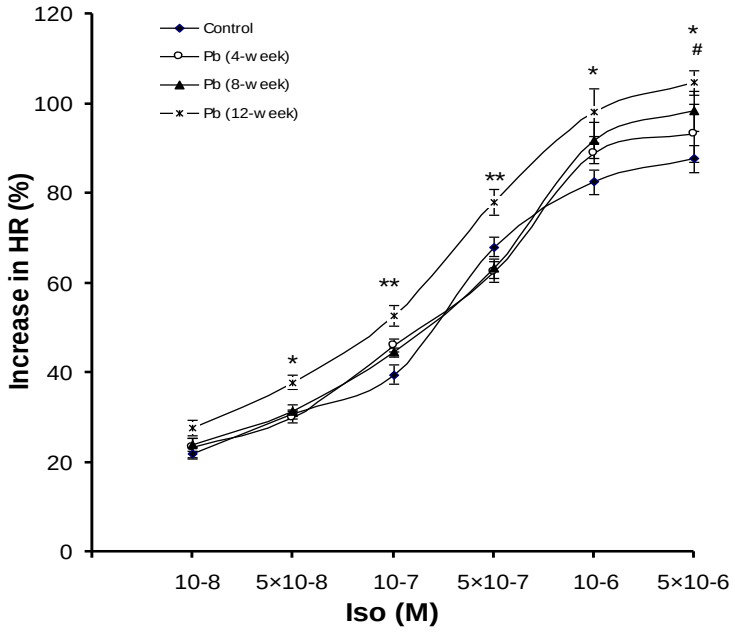

Figure 1. The chronotropic response of isolated heart to isoproterenol (Iso, M) in control and lead-treated (Pb) rats (Data presented as mean ± SEM, n=6; #,*P<0.05, **P<0.01).
(In all graphs, #: Differences between control and 8-week lead-treated rats, *: Differences between control and 12-week lead-treated rats.)

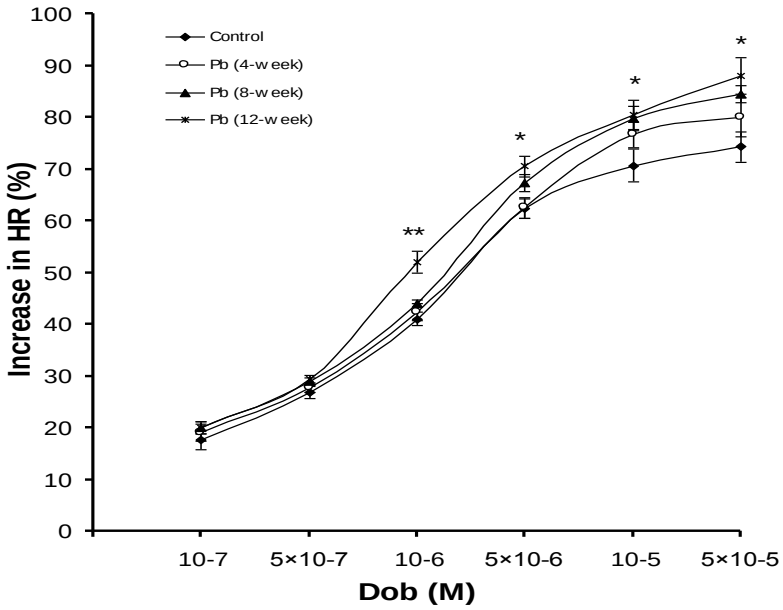

Figure 2. The chronotropic response of isolated heart to Dobutamine (Dob, M) in control and lead-treated (Pb) rats (Data presented as mean ± SEM, n=6; *P<0.05).

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 exposure to low amounts of lead could not change either the contractility or sensitivity of the isolated heart to β-adrenergics. However, contractile responses of isoproterenol in 8- and 12-week lead-treated groups were significantly increased (P<0.05 and P<0.01) (figure3).

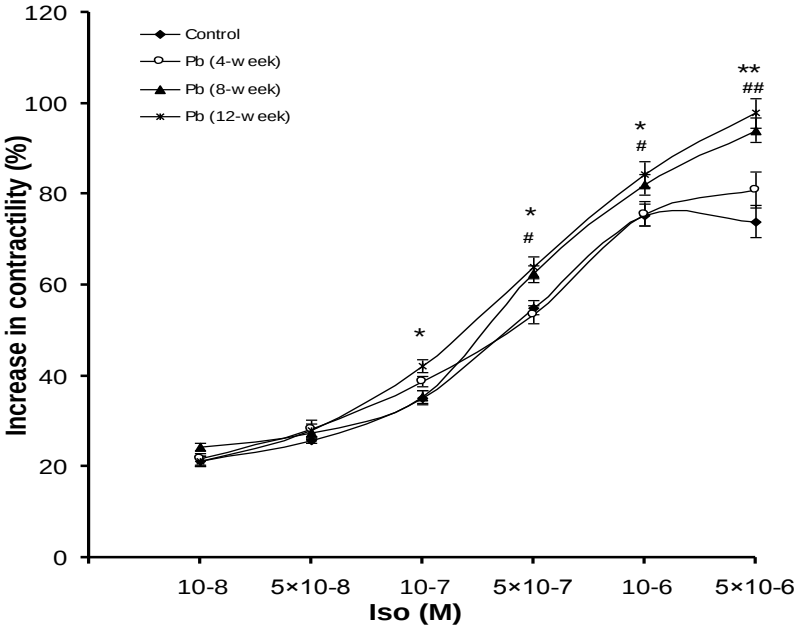

Figure 3. The inotropic response of isolated heart to isoproterenol (Iso, M) in control and lead-treated (Pb) rats (Data presented as mean ± SEM, n=6; #,*P<0.05; ##, **P<0.01).

Similar findings were obtained with dobutamine (figure 4). Dobutamine contractile response 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 effect. Moreover shifting contractile curves to upward and left side shows the augmentation of the β-adrenoceptors sensitivity by lead.

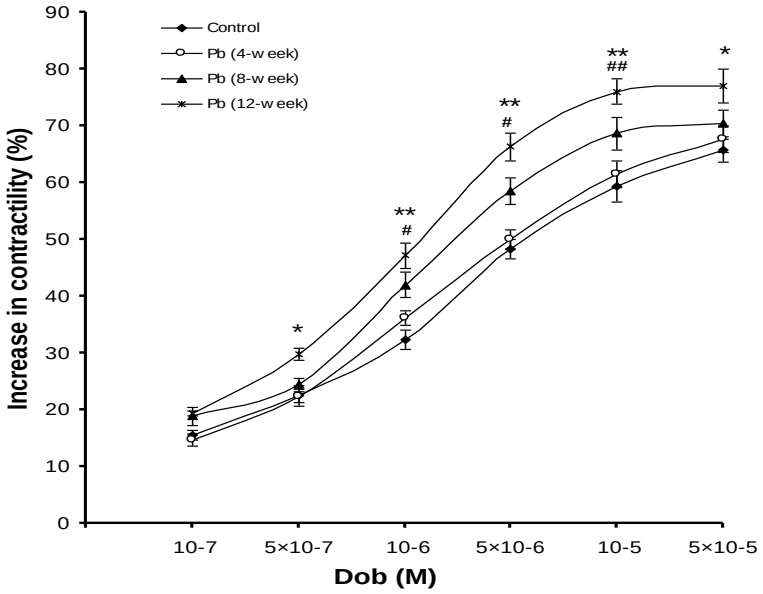

Figure 4. The inotropic response of isolated heart to Dobutamine (Dob, M) in control and lead-treated (Pb) rats (Data presented as mean ± SEM, n=6; #,*P<0.05; ##, **P<0.01).

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

Discussion

In the present study, the 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 beating 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 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 in the period of 4 weeks did not alter the responsiveness of isolated heart to catecholamines.

Alteration of cardiovascular responsiveness to β–adrenergics has been demonstrated in several models of hypertension. In previous studies, it has been explained that an increase in vascular contractile responsiveness to adrenergic agonists is shown in lead-induced hypertension (Heydari *et al*., 2006; Sharifi *et al*., 2004). However, there are controversial results among studies (Carmignani *et al*., 2000; Bertel *et al*., 1978). Pathogenesis of lead-induced hypertension could be explained by increase in rate and contractility of the heart.

Increased chronotropic (Skoczynska *et al*., 1986) and inotropic (Carmignani *et al*., 2000) effects of isoproterenol that augment β-adrenoceptors activity *in vivo* in the heart, were noted in some previous studies. However, a diminished chronotropic response to isoproterenol is also reported in a lead-poisoned patient (Bertel *et al*., 1978), as well as a decreased inotropic effect of this drug is explained in the rat isolated hearts perfused with a solution containing lead acetate (Kopp and Barany, 1980), which disagree with our results. The lead dosage used by these investigators was much greater than the dosage used in our experiment. Therefore, 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 (Heydari *et al*., 2006; Skoczynska *et al*., 2001).

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 a higher affinity of agonist to its receptors or an augmented activity of adenylate cyclase and as a result, an increase in the intracellular concentration of cAMP and Ca^{2+}. However, Chang and his colleagues (Chang *et al*., 1997) reported that lead exposure results in a reduction of cardiovascular β-adrenoceptor density and a diminution of cAMP accumulation in brain (Tsao *et al*., 2000). 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, 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 been 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 (Boscolo and Carmignani, 1988; Iannaccone *et al*., 1981). 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 (Carmignani *et al*., 2000). Therefore, although the amounts of cAMP are likely reduced in the presence of lead (Tsao *et al*., 2000), but the β_1-adrenoceptor activation (sensitization) by lead will be able to elevate cAMP activity even more than in the absence of lead exposure, despite a likely lower β_1-adrenoceptor density (Chang *et al*., 1997).

Lead, directly or by augmenting the sensitivity of cardiovascular system to cathecholamines, acts on vascular smooth muscle cells to increase vascular tonicity (Heydari et al., 2006). This, along with the lead-induced increase in cardiac inotropism and chronotropism elevate systolic blood pressure. These conditions in a prolonged period may raise the cardiac work and cause other complications such as heart failure.

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