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آموزش مهارت های کاربردی در تدوین و چاپ مقاله
ACUTE LEAD EXPOSURE AND CONTRACTION OF RAT ISOLATED AORTA INDUCED BY D1-DOPAMINERGIC AND ALPHA-ADRENERGIC DRUGS

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Background/Objective: In the present study, the effect of acute lead exposure in the presence and absence of dopamine or alpha (α)-adrenoceptor agents on contractile response of rat isolated thoracic aorta was studied.

Methods: Male Wistar rats were used in all experiments. Thoracic aorta was carefully removed, cleaned, and cut into 2-mm thick rings. The rings were mounted for measurement of isometric contractions in a tissue bath containing 10 mL of Kreb’s solution at 37 – 38 °C. The following drugs were used: lead chloride, dopamine, phenylephrine, prazosin, clonidine, yohimbine, and SCH23390. One-way analysis of variance (ANOVA) and Student’s t-test were used for statistical analyses. P < 0.05 was considered significant.

Results: The α1-adrenoceptor antagonist (prazosin), α2-adrenoceptor antagonist (yohimbine), or dopamine D1 receptor antagonist (SCH23390), did not elicit any response. Combination of lead with dopamine, phenylephrine, or clonidine did not show any potentiation. SCH23390, prazosin, and yohimbine decreased the contraction induced by lead. SCH23390 decreased the contraction induced by dopamine, or lead plus dopamine. Prazosin reduced the contraction induced by phenylephrine or lead plus phenylephrine. Yohimbine attenuated the response induced by phenylephrine or lead plus clonidine.

Conclusion: α1, α2, and D1 dopamine receptor mechanisms could have a role in lead-induced contraction.

Keywords: Alpha (α)-adrenergic • dopaminergic • lead exposure • rat • thoracic aorta rings

Introduction

Lead is a toxic metal ion that is involved in the pathogenesis of some types of hypertension.1 – 4 There is, however, no conclusive evidence about the mechanisms involved in the effects of lead.6, 7 It has also been proposed that lead does not directly contract proteins and it has no influence on blood vessel contraction induced by phospholipid messenger pathway or relaxation due to nitric oxide and cAMP. At higher doses (10^-4 mol/L), it may increase the contractions induced by low concentrations of calcium. While, acute short-term lead exposure exerts a direct effect on blood vessel contraction and affects blood pressure regulation,8 – 10 short-term incubation of aorta rings with either high or low concentrations of lead failed to modify the response to different vasodilator or vasoconstrictor agonists.11 Moreover, the effects of lead on central and peripheral catecholaminergic pathways and autonomic nervous system have not been specifically related to the blood lead levels found in chronically lead-exposed workers12 or population living in urban and extraurban areas.13, 14 It has, however, been shown that high levels of lead are able to increase plasma catecholamine levels and to reduce both the number of the vascular β-adrenoceptors and β-adrenoceptor-induced increase in cAMP concentration in the
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Materials and Methods

Animals
Male Wistar albino rats, weighing 150 – 250 g were used in all experiments. Rats were housed 8 per cage in an animal room with 12/12hr light/dark cycle, at 22 ± 2° C. The animals had access to a standard cube diet and water ad libitum. The experimental protocol was approved by the Research and Ethical Committee of V.P. Chest Institute, University of Delhi (Res. No. M/C/Elect/2000 – 01, 12 January, 2001).

Measurement of contractions
The experiments were performed in an organ bath setup for studying isolated blood vessel preparations. Thoracic aorta was carefully removed, cleaned, and cut into 2-mm thick rings. Two rings were prepared and used from each rat thoracic aorta. The rings (evaluated with endothelium) were mounted for measurement of isometric contraction in a tissue bath containing 10 mL of Kreb’s solution at 37 – 38°C. The solution in the organ bath (with a constant pH) contained Kreb’s bicarbonate, gased with 5% CO 2 and 95% O 2, of the following composition, in g/L: NaCl 7; KCl 0.35; MgSO 4 0.3; KH 2PO 4 0.16; NaHCO3 2.1; D-glucose 2, and CaCl 2 (2.5 mL of 1 M solution, drop by drop). The isometric contraction of aorta ring was measured by an isometric transducer (F-50) and recorded on a PMP-4A physiograph (Narco Biosystems Inc., USA). Resting tension was adjusted to 2 g. After equilibration for 1 hr, the tissue was contracted by phenylephrine (10 -6 M) before carrying out the experiments. The tissues were washed, exposed to the Kreb’s solution, and allowed to relax to baseline value. Aorta responses to drugs were tested over a range of 10 -13 – 10 -4 M. Each cumulative concentration of drugs per se or in the presence of any other drug was tested on 8 thoracic aorta rings taken from different rats.

Drugs
The following drugs were used: lead chloride (Merck, Germany), dopamine hydrochloride (TTK Health Care Limited, India), phenylephrine hydrochloride, prazosin hydrochloride, clonidine hydrochloride and yohimbine (Sigma, Poole, UK), and SCH23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-olaleate; Research Biochemical Inc., USA). The drugs were dissolved in normal saline and prepared immediately before use.

Statistical analysis
One-way analysis of variance (ANOVA) and Student’s t-test were used for statistical analyses. P < 0.05 was considered significant.

Results
The effects of phenylephrine, clonidine, dopamine, prazosin, yohimbine, and SCH23390 on contraction of rat isolated aorta
In four series of experiments, each consisting of eight aorta rings, cumulative concentrations of alpha (α 1)-adrenoceptor agonist, phenylephrine (LogED 50 = -6.0 ± 0.15); α 2-adrenoceptor agonist, clonidine (LogED 50 = -6.4 ± 0.44); dopamine (LogED 50 = -5.8 ± 0.43); and lead (LogED 50 = -9.0 ± 0.24) induced a dose-dependent contraction of the isolated rat aorta.

In three series of experiments, each consisting of eight aorta rings, the cumulative concentrations of α 1-adrenoceptor antagonist, prazosin (LogED 50 = not applicable); α 2-adrenoceptor antagonist, yohimbine (LogED 50 = not applicable); and dopamine D 1 receptor antagonist, SCH23390 (LogED 50 = not applicable) did not alter the contractile response of isolated rat aorta.

In four series of experiments, each consisting of eight aorta rings, four concentrations of lead (10 -13 , 10 -11 , 10 -9 , and 10 -4 M) in combination with a cumulative concentration of dopamine (10 -13 – 10 -4 M) did not show any potentiation.

The effects of dopamine and SCH23390 or phenylephrine and prazosin on lead-induced contraction of the isolated rat aorta
In the first experiment, aorta tissues (eight in each group) were exposed to lead (10 -4 M) for 10 min. The aorta tissues were then exposed to a cumulative concentration (10 -13 – 10 -4 M) of SCH23390, prazosin, or yohimbine and the results were compared with that of lead when used alone.
All the antagonists abolished the lead-induced contraction of aorta (Figure 1).

In the second experiment, four groups of aorta tissues (n = 8) were exposed either to a cumulative concentration (\(10^{-13} \text{ to } 10^{-4} \text{ M}\)) of dopamine, or dopamine in the presence of lead, SCH23390, or lead plus SCH23390. The results indicated that the combination of lead with dopamine did not show a potentiated influence on the contractile response of aorta. The contraction induced by higher doses of dopamine or dopamine plus lead was reduced by SCH23390 (Figure 2).

In the third experiment, four groups of aorta tissues (n = 8) were exposed either to a cumulative concentration (\(10^{-13} \text{ to } 10^{-4} \text{ M}\)) of phenylephrine, or phenylephrine in the presence of lead, prazosin, or lead plus prazosin. It has been shown that a combination of lead with phenylephrine did not show any potentiation of the contractile response of aorta. The contraction induced by higher doses of phenylephrine or phenylephrine plus lead was reduced by prazosin (Figure 3).

In the fourth experiment, four groups of aorta tissues (n = 8) were exposed either to a cumulative concentration (\(10^{-13} \text{ to } 10^{-4} \text{ M}\)) of clonidine, or clonidine in the presence of lead, yohimbine, or lead plus yohimbine. Results indicated that the combination of lead with clonidine did not show a potentiated influence on the contractile response of aorta. The contraction induced by lead, clonidine, or lead plus clonidine was reduced by yohimbine (Figure 4).

**Discussion**

Adrenergic drugs are able to elicit contraction of arterial smooth muscles. In the present study, \(\alpha_1\)-adrenoceptor agonist (phenylephrine) and \(\alpha_2\)-adrenoceptor agonist (clonidine) caused contraction of rat aorta. The response induced by phenylephrine or clonidine was decreased by prazosin and yohimbine, respectively. In agreement with previous results, \(\alpha\)-adrenoceptor stimulation induced contraction of the aorta. There are reports that activation of \(\alpha_3\)-adrenoceptors on endothelial cells stimulates the release of nitric oxide (NO), an action that would tend to attenuate vasoconstriction produced by activation of postjunctional vascular \(\alpha_1\)-adrenoceptors. Therefore, the contraction induced by clonidine may be due to the response of \(\alpha_2\)-adrenoceptor. It has also been proposed that \(\alpha_2\)-adrenoceptor activation inhibits adenylate cyclase activity in the vascular myocytes, leading to a higher amounts of Ca\(^{2+}\) and thus, allowing the entry of Ca\(^{2+}\) ions through voltage-gated Ca\(^{2+}\) channels, and consequent contraction.
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Dopamine also elicited contraction of aorta in the present study. The drug is thought to produce contraction in peripheral vessels through stimulation of α-adrenergic receptors and vasodilation primarily through stimulation of β-adrenergic receptors. Vasoconstriction is thought to predominate at higher doses of dopamine in the peripheral circulation. The results of some studies have demonstrated that dopamine, at both low and high doses, when administered as an intravenous bolus to healthy conscious dogs, affects the coronary circulation directly, primarily through stimulation of α- and possibly dopaminergic receptors. Dopamine increases cAMP concentration through D_1 dopamine receptor stimulation. These results indicate that dopamine D_1 receptor antagonist SCH23390 can reduce the dopamine-induced contraction and therefore, the response of dopamine may be due to D_1 receptor activation which is similar to α-adrenoceptor agonists; the action may be mediated by an increase in cAMP levels.

The present data also showed that higher concentrations of lead induced contraction of the rat aorta. This is in agreement with previous reports indicating that lead just at its highest concentration (10^{-4} M), increases contractions to calcium at all submaximal calcium concentrations. Studies in vitro have shown that lead can block postsynaptic adenylate cyclase activity. The enzyme may be coupled with dopamine receptors and alterations in their functions may be elicited through cAMP levels. This cannot account for the lead effect, since D_1 dopamine receptor stimulation, which increases cAMP levels, induces contraction of the vascular smooth muscles. It can be proposed that cAMP may exert a modulatory effect.

The endothelium plays an inhibitory role in contractions in rat femoral arteries by releasing NO. Although lead does not destroy the monolayer of endothelial cells, the metal may exhibit its noxious effects in the repair process of the vascular endothelium. Lead inhibits NO synthase (NOS). The lead-induced contraction may, therefore, be elicited through this mechanism.

Lead appeared to increase both sympathetic nerve activity by central mechanisms (thus increasing the plasma noradrenaline and adrenaline levels) and cAMP-dependent availability of Ca^{2+} ions for contractile mechanisms in the vascular and cardiac myocytes (also through an increased vascular α_2- and myocardial β_1-adrenoceptor reactivity). Any of these mechanisms may account for the lead-induced aorta contraction.

In the present study, combination of neither phenylephrine and clonidine nor dopamine altered the lead effect. However, prazosin, yohimbine, and SCH23390 decreased the lead response, which
may suggest that adrenergic and dopaminergic receptor mechanisms may be involved in the lead effect or at least a common second messenger mechanism could mediate the lead and aminergic responses.

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References

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