

# Effects of Lead on Vascular Reactivity

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Considerable controversy exists concerning the possible role of lead in the etiology of human hypertension. In animal studies, there is convincing evidence that lead alters cardiovascular responsiveness; rats drinking water containing 100 ppm lead develop a chronic, significant 15 to 20 mm Hg elevation in systolic blood pressure. Pressor responsiveness to catecholamines is enhanced in animals chronically exposed to lead, and the responsiveness of isolated vascular smooth muscle to adrenergic agonists is increased in rats with lead-induced hypertension. Experimental evidence suggests that alterations in the cellular mechanisms that regulate intracellular calcium concentration may contribute to the abnormal vascular function in lead-induced hypertension. Recent work in our laboratory indicates that increased vascular reactivity in genetic hypertension is associated with altered activity of the protein kinase C branch of the calcium messenger system. Contractile responses to lead in rabbit mesenteric artery are potentiated by activators (phorbol esters) of this enzyme complex, and a selective inhibitor of protein kinase C inhibited contractions induced by lead. Based on these results, it is proposed that a cellular component of the action of lead to increase vascular reactivity may relate to the role of protein kinase C in smooth muscle contraction.

## Introduction

Recent evidence indicates that occupational and/or environmental lead exposure may be related to increased arterial blood pressure (1-3). Furthermore, studies on experimental animals have demonstrated that low-level lead exposure produces hypertension (1,4-10). As most forms of clinical and experimental hypertension are characterized by increased peripheral vascular resistance and abnormal vascular reactivity (11), it is not surprising that alterations in the vasculature have been proposed as mechanistic components of the increased blood pressure due to lead exposure (1-8). This brief review will summarize evidence bearing on the nature of functional vascular changes in lead-induced hypertension and then cite evidence suggesting that the effects of lead are related to altered cellular calcium metabolism. Finally, new experimental evidence will be presented indicating that these vascular abnormalities may be due to a specific action of lead on the protein kinase C branch of the calcium messenger system. Readers interested in a more complete review of cardiovascular changes that accompany lead exposure are referred to other recent publications (1,12).

## Lead Exposure and Vascular Reactivity

Several lines of evidence suggest that increased vascular reactivity to pressor agents may be one of several factors that contribute to hypertension (11). Animal models of genetic, mineralocorticoid and renal hypertension, and human essential hypertension are characterized by abnormal vascular responsiveness to a number of physiological and nonphysiological stimuli (10,13,14). The results of different studies are highly variable, but it is certain that structural and functional changes in the vascular wall contribute to abnormal constrictor and dilator responses. Structural changes that characterize hypertension have been emphasized by Folkow and co-workers (13), who present evidence that the altered geometry of the arterial vascular wall provides a mechanical advantage to increase arterial pressure during vasoconstriction. Structural vascular changes also limit minimal flow resistance and are primarily an adaptive response to the elevated blood pressure in the hypertensive state. The functional vascular changes that characterize hypertension are varied and complex, but the major observation is that the activity of the smooth muscle is enhanced during constrictor events and reduced during dilator events (11,14).

In rats fed lead, pressor responses to norepinephrine and angiotensin II have been reported to be either

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increased or unchanged compared to control, untreated rats (7,10,15). Because these studies on intact animals are confronted with the interpretive problem of differentiating a vascular from a cardiac response, other investigators have characterized vascular actions of dietary lead in isolated systems. Webb et al. (8) observed that helical strips of tail arteries from lead-treated rats displayed a greater force-generating ability in response to methoxamine and norepinephrine than arterial strips from control rats (Fig. 1). This altered contractile responsiveness to adrenergic agonists in lead-treated animals was specific, as contractions to depolarizing concentrations of potassium in arteries from lead-treated rats did not differ from those in arteries from control rats. The enhanced contractile responses to methoxamine and norepinephrine were not due to alterations in the activity of adrenergic nerve endings in the arterial wall, nor were the responses a function of altered  $\beta$ -adrenergic activity in the smooth muscle. Furthermore, the change in maximum force-generating ability was not an acute alteration, as tail arteries from control rats did not exhibit abnormal responsiveness to  $\alpha$ -adrenergic agonists when exposed to lead in the physiological buffer system.

Studies by other investigators (16–19) have documented that lead can act in an acute way to alter vascular reactivity. Prentice and Kopp (16) observed that rat hearts perfused with 30  $\mu$ M lead had reduced coronary blood flow compared to untreated hearts, presumably because lead directly constricted the coronary arteries or interfered with metabolic stimuli that

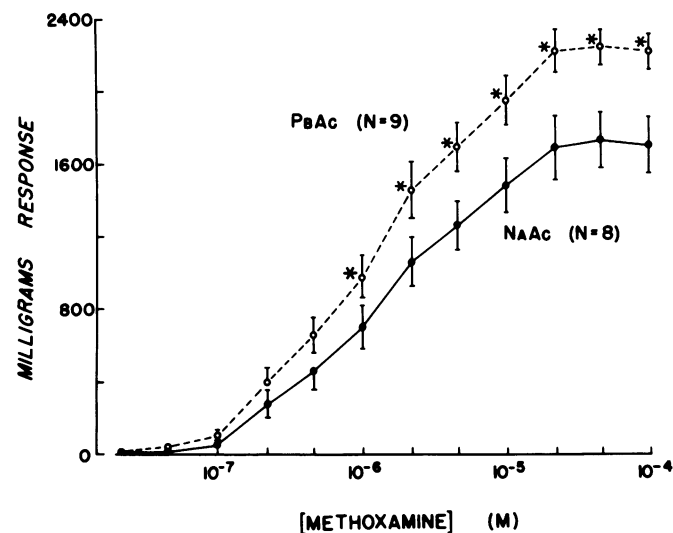


FIGURE 1. Concentration-response curve to methoxamine. Helical strips of tail artery from rats treated with sodium acetate (NaAc) or lead acetate (PbAc) were made to contract in response to the cumulative addition of methoxamine to the muscle bath. Arterial strips from PbAc-treated rats developed more force in response to all concentrations of methoxamine than did those from control rats. Values are means  $\pm$  SEM. The number of animals in each group is in parentheses. Asterisk (\*) indicates a statistically significant difference between rat groups ( $p < 0.05$ , unpaired  $t$ -test). Reproduced by permission from Webb et al. (8).

normally cause vasodilatation. In rabbit aorta, lead causes contraction when added directly to the muscle bath (17), and contractile responses to calcium in depolarized tail artery segments are augmented by exposure to lead in the buffer system (18,19).

A unique and unusual response that characterizes vascular reactivity in lead-treated rats was observed by Webb et al. (8). These investigators observed that tail artery strips from lead-treated rats exhibited a greater incidence of spontaneous contractile activity than did those from control rats. These spontaneous contractions were only apparent during the equilibration period of the experiment and were probably due to the leakage of norepinephrine from nerve endings in the vessel wall.

These functional studies in intact animals and on isolated vascular preparations indicate that lead alters the contractile activity of smooth muscle and therefore may reduce blood vessel caliber, contributing to the hypertensive state in animals exposed to the heavy metal.

## Calcium and Vascular Effects of Lead

Because the contractile state of vascular smooth muscle varies in proportion to the concentration of calcium in the cytoplasm, several investigators have tested the hypothesis that increased vascular reactivity in lead-induced hypertension is due to the ability of lead to alter cellular calcium metabolism (8,17–19). Two types of studies have been performed: a) measurement of contractile responses in calcium-free solution or after treatment with drugs that block membrane channels for calcium; and b) quantification of the movement of radiolabeled calcium in blood vessels treated with lead.

Calcium-free solution attenuates contractile responses to lead in aortic smooth muscle from rabbits, suggesting that activator calcium is important for the effect of the heavy metal (17). Methoxamine-induced contractions in tail arteries from rats made hypertensive by low lead exposure are less sensitive to the inhibitory effects of the calcium channel blocker, D-600, than similar contractions in arteries from normotensive rats (8) (Fig. 2). These results were interpreted as indicating that there is an enlarged intracellular calcium pool in arteries from lead-treated rats that maintained the contractile effect of methoxamine even in the face of calcium channel blockade. Supportive evidence for this interpretation was that tail arteries from lead-treated rats relaxed at a slower rate after removal of methoxamine from the tissue bath than control arteries. Presumably, the rate of relaxation reflected the removal of activator calcium from the cytoplasm, and this process was abnormal in arteries from lead-treated rats. (An alternative explanation for these results will be covered in the following section which deals with the protein kinase C branch of the calcium messenger system.)

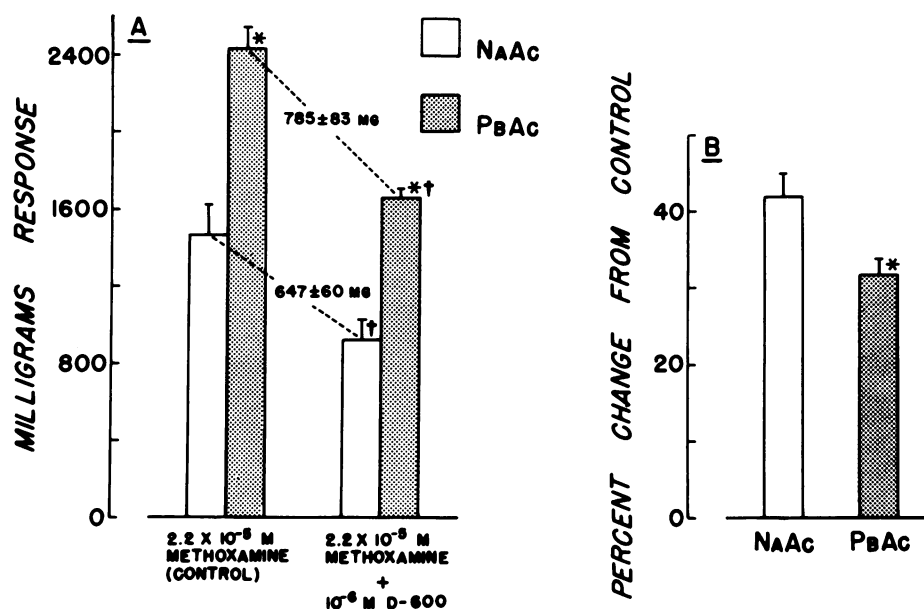


FIGURE 2. D-600 and contraction induced by methoxamine. In the absence of D-600, arterial strips from PbAc-treated rats developed more force than those from control rats. In the presence of D-600, contractile responses in both groups of rats were depressed, but the absolute magnitude of depression was similar for both groups (A). Normalization of contractile responses in the presence of D-600 to their respective control responses indicated that arterial strips from PbAc-treated rats had a smaller component of total contraction that was sensitive to the calcium channel blocker (B). Values are the mean  $\pm$  SEM for five rats in each group. Asterisk (\*) indicates a statistically significant difference between rats treated with NaAc and those treated with PbAc ( $p < 0.05$ , unpaired *t*-test). Dagger (†) indicates a statistically significant difference between the controls and treatment with  $10^{-6}$  M D-600 within each group of rats ( $p < 0.05$ , paired *t*-test). Reproduced by permission from Webb et al. (8).

Piccinini et al. (18) observed that *in vitro* treatment with lead increased the tissue content of radioactive calcium in the rat tail artery. Further, the half-time of the slow component of the radiolabeled calcium washout (which probably reflects the extrusion of calcium from the cytosol) was attenuated in the presence of lead. The authors suggested that intracellular calcium binding sites are involved in the action of this metal in vascular smooth muscle.

Studies on nonvascular tissues also support the concept that lead interferes with cellular calcium metabolism (1). This work on vascular and nonvascular tissue is compatible with current works on other experimental models of hypertension, where it has been demonstrated that alterations in calcium transport processes may contribute to altered cellular events leading to elevated arterial pressure (11,14).

## Vascular Actions of Lead and Protein Kinase C Activators

Current concepts about cellular and molecular events underlying smooth muscle contraction indicate a role for polyphosphoinositide turnover and protein kinase C activity (20–22). In response to certain hormones and neurotransmitters, phosphatidylinositol 4,5-*bis*-phosphate of the plasma membrane is hydrolyzed by phospholipase C. One product of this hydrolysis, inositol 1,4,5-trisphosphate, has been proposed

to cause a release of calcium from intracellular membrane stores (21). Another product of receptor-mediated phosphoinositide metabolism is diacylglycerol, which activates the membrane-bound, calcium/phospholipid-dependent protein kinase C, resulting in the phosphorylation of structural and regulatory proteins involved in contraction (17). Protein kinase C activators, such as the tumor-promoting phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), have been used to study the cellular actions of protein kinase C in smooth muscle (23–26).

Recent studies in our laboratory have characterized vascular responsiveness to lead following incubation with activators and inhibitors of protein kinase C (Figs. 3 and 4). Isolated strips of rabbit mesenteric artery contracted in response to the cumulative addition of lead ( $10^{-9}$ – $10^{-5}$  M) to the muscle bath, and this contractile effect was potentiated by pretreatment with 0.3  $\mu$ M TPA (Fig. 3). We propose that an important action of lead may be to alter the activity of protein kinase C and therefore modify subsequent cellular phosphorylation events leading to contraction. Evidence in support of this hypothesis is that contractile responses to lead were attenuated by the selective inhibitor of protein kinase C, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (27) (Fig. 4). This inhibitor did not alter contractile responses to high concentrations of potassium ion, suggesting that the intervention may be selective for protein kinase C-mediated contraction.

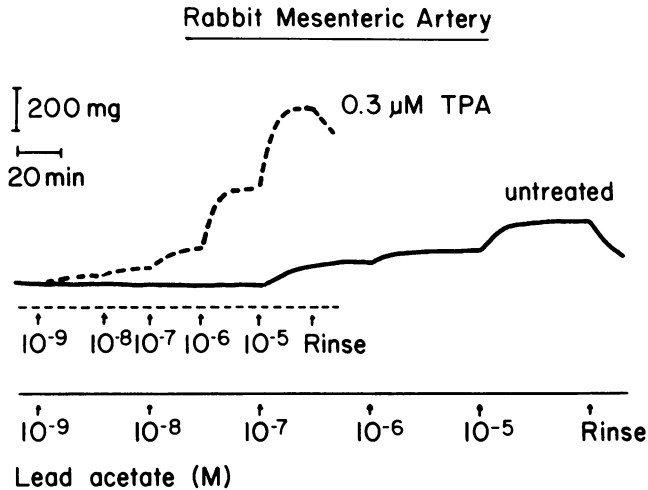


FIGURE 3. Contractile responses to lead acetate in the presence and absence of a phorbol ester. A helical strip of mesenteric artery from a rabbit was made to contract in response to lead acetate ( $10^{-9}$  to  $10^{-5}$  M) before and after treatment with  $0.3 \mu\text{M}$  TPA (added 5 min before the addition of lead acetate). Contractile responses were greater in the presence of the phorbol ester than in the untreated, control condition. The concentrations of lead acetate are noted on separate scales because force development to lead acetate in the presence of TPA (---) was faster than in its absence (—). Reproduced by permission from Chai and Webb (unpublished observations).

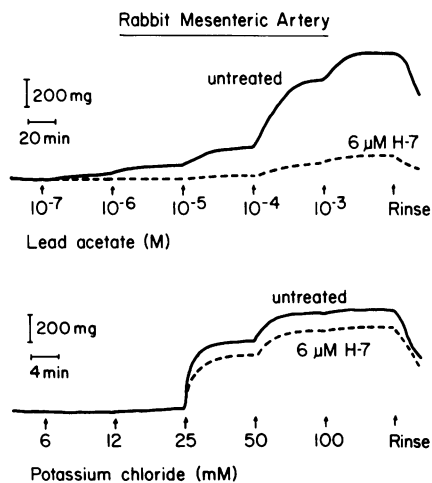


FIGURE 4. Effect of an inhibitor of protein kinase C (H-7) on contractile responses to lead acetate and potassium chloride. Helical strips of rabbit mesenteric artery were made to contract in response to lead acetate ( $10^{-7}$  to  $10^{-3}$  M, top tracings) or potassium chloride (6–100 mM, bottom tracings) in the presence (---) or absence (—) of  $6 \mu\text{M}$  H-7. Contractile responses to lead acetate in the presence of the inhibitor of protein kinase C were greatly attenuated compared to the control, untreated condition, whereas contractile responses to depolarizing concentrations of potassium in the presence of the inhibitor were only slightly depressed compared to the untreated condition. Reproduced by permission from Chai and Webb (unpublished observations).

Recent work by Goldstein and co-workers (28) offers biochemical evidence that lead alters protein kinase C activity in homogenates of rat brain microvessels.

These investigators assayed for protein kinase C activity using exogenous lysine-rich histone as a substrate. Calcium and lead stimulated the activity of protein kinase C, and lead was more potent than calcium in increasing the activity of the enzyme. In addition, it was observed that lead may have a synergistic action with calcium in the activation of protein kinase C in cerebral microvessels.

Studies on blood vessels from genetically hypertensive rats have also documented an enhanced contractile response to protein kinase C activators compared to normotensive values (23). As the stimulation of this enzyme is an important step in membrane activation by certain hormones and neurotransmitters (20), it is possible that altered protein kinase C activity contributes to increased vascular reactivity in hypertension. It appears that a cellular component of the action of lead in vascular smooth muscle may relate to the activity of protein kinase C.

This work was supported by a grant (HL-27020) from the National Institutes of Health.

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