Perinatal Lead Exposure Affects Nitric Oxide and Cyclooxygenase Pathways in Aorta of Weaned Rats

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Perinatal Pb exposure may modulate arterial tone through nitric oxide (NO) and cyclooxygenase products. To investigate this, Wistar dams received 1000 ppm of Pb or sodium acetate (control) in drinking water during pregnancy and lactation. Curves were constructed in phenylephrine-precontracted intact and/or denuded rings of thoracic aortas of weaned (23-day-old) male pups from their responses to N\textsuperscript{ω}-nitro-L-arginine methyl ester (L-NAME, NO synthase inhibitor) and ACh in the absence or presence of indomethacin (10\textsuperscript{-5} M, cyclooxygenase inhibitor) or L-NAME (3×10\textsuperscript{-5} M and 3×10\textsuperscript{-7} M). Blood lead concentration and systolic blood pressure (SBP) were higher in intoxicated than control pups (blood lead μg/dl: control < 3.0, Pb 58.7 ± 6.5*; SBP mmHg: control 111.4 ± 2.3, Pb 135.5 ± 2.4*). In L-NAME–treated rings maximal responses increased in Pb-exposed rats, and were higher in intact than in denuded aortas (contraction [% of phenylephrine] intact: control 184.3 ± 23.7, Pb 289.1 ± 18.3*; denuded: control 125.1 ± 4.5, Pb 154.8 ± 13.3*). ACh-induced relaxation in intact aortas from Pb-exposed rats presented a rightward shift in L-NAME presence (EC50 × 10\textsuperscript{-7} M: control 1.32 [0.33–5.18], Pb 4.88 [3.56–6.69]* but moved left under indomethacin (EC50 × 10\textsuperscript{-7} M: control 8.95 [3.47–23.07], Pb 0.97 [0.38–2.43]*). \*p < 0.05 significant relative to the respective control; N = 7–9. Endothelium removal abolished ACh-induced relaxation. Perinatal Pb exposure caused hypertension associated with alterations in the production and/or release of basal and stimulated endothelium-derived relaxing factors—NO and constricting cyclooxygenase products. These findings may help explain the contribution of NO and cyclooxygenase products to the etiology and/or maintenance of Pb-induced hypertension and could ultimately lead to therapeutic advantages in plumbism.

Key Words: metals; developmental toxicology; cardiovascular system; endothelial factors; perinatal intoxication; weaned rats.

Lead (Pb) acquires greater importance every year as both an industrial and environmental hazard. This has led to concern about the impact of increased Pb burden on blood pressure and cardiovascular diseases.

Some epidemiological data are compatible with the idea that Pb exposure plays a role in the development of the arterial hypertension in persons occupationally exposed to Pb (Cooper and Gaffey, 1975) as well as in the general population (Bost et al., 1999). Nowack et al. (1993) reported a biphasic effect of Pb on blood pressure of rats.

In adult rats, the arterial hypertension induced by Pb exposure during postnatal life is characterized by an increase in vascular reactivity to catecholamines (Skoczynska et al., 1986), and an increase in plasma noradrenaline level as well as a decrease in beta-adrenergic receptors (Tsao et al., 2000). Contrarily, Purdy et al. (1997) reported no aorta reactivity alteration to either vasoconstrictors (noradrenaline and phenylephrine) or vasodilators (acetylcholine, ACh and sodium nitroprusside, SNP) associated with Pb-induced hypertension in rats.

Most studies have focused on the concept that this heavy metal interacts with and (1) regulatory processes involving calcium ion, cyclic guanosine monophosphate as second messengers, and protein kinase C (Khalil-Manesh et al., 1993; Watts et al., 1995); and (2) rennin–angiotensinn–aldosterone, kallikreinn–kinin, and other autacoidal (e.g., endothelin) and transductional systems (e.g., nitric oxide, NO) (Khalil-Manesh et al., 1993; 1995) reported a biphasic effect of Pb on blood pressure of rats.

Several diseases developing during adulthood were probably determined during early stages of life, under the effect of exposure or preferential maternal diet during pregnancy. Although a great deal of progress has been made with regard to neurochemical, behavioral, and other alterations induced by perinatal exposure to Pb (Dearth et al., 2002; Lasley et al., 1992; Moreira et al., 2001), the effects of Pb exposure during pregnancy and lactation on cardiovascular system remain to be completely clarified.

Thus, the present study aimed to evaluate the impact of perinatal exposure to Pb on NO and cyclooxygenase pathways...
modulation of arterial tone. These studies advance understanding of the cardiovascular effects of Pb exposure during pregnancy and lactation and could aid in the development of proper therapies for cardiovascular diseases caused by plumbism.

MATERIAL AND METHODS

Animals and treatment. Wistar rats were obtained from University of São Paulo facilities and used as the parent generation. The animals were mated at the age of 90 days (two females and one male per cage). On pregnancy day 0 (determined by the presence of sperm in vaginal smears), the dams were divided into nonintoxicated or Pb-exposed groups and were housed singly. The drinking water of dams was altered with 1000 ppm Pb (as Pb acetate) or sodium acetate to equalize acetate exposure for the groups. The Pb exposure regimen was chosen based on previous studies (Berry et al., 2002; Kitchen and Kelly, 1993; Ma et al., 1997; Reinholz et al., 1999). To prevent the formation of Pb precipitate, 0.5 ml of glacial acetic acid was added while stirring to prepare 1000 ml of both solutions (sodium acetate and Pb). The treatment lasted throughout pregnancy and lactation. At birth, the number of pups per litter was recorded and after which all litters were culled to eight pups. Whenever possible, only male rats were kept within the litter and females were kept just to maintain equal litter sizes. Pb-exposed rats were weaned at 21 days of age and evaluated at 23 days of age. Aged matched-controls received sodium acetate during the same periods of Pb exposure. Maternal body weights were measured on pregnancy day 0, the day before delivery, after delivery and at weaning. Pup weights were recorded at birth and at weaning.

Lights in the animal room were set on 12:12-h cycle with temperature maintained at 22 ± 1°C. The animals were fed with regular lab chow. Animal procedures were in accordance with the principles and guidelines of the National Council for Control of Animal Experimentation.

Measurement of blood pressure. Systolic blood pressure (SBP), in conscious rats, was determined using tail-cuff plethysmography (Narco Bio-Systems, Inc., Houston, TX), on postnatal day 22. The rats were prewarmed as micrograms per deciliter. The detection limit was 3 μg/kg. Measurement of blood pressure throughout pregnancy and lactation. One ring served as control, whereas the other was examined in phenylephrine-precontracted intact aortic rings at concentrations that induced 60–80% of the maximum effect in the absence and presence of different concentrations of L-NAME (3 × 10–7 M, more selective for endothelial NOS (eNOS) at this concentration, and 3 × 10–6 M, unspecific for NOS subtypes) (Wu and Yen, 1999). Removal of endothelium abolished the response to ACh.

Response to exogenous NO donor. Cumulative concentration effect curves for ACh, an endothelium-dependent relaxant agent, were examined in phenylephrine-precontracted intact aortic rings at concentration that induced 60–80% of the maximum effect in the absence and presence of two different concentrations of L-NAME (3 × 10–7 M, more specific for NOS; Cignarella et al., 2000; Rees et al., 1999).

RESULTS

Body Weight, Number of Pups, Blood Lead Concentration, and Blood Pressure

The Pb regimen employed in the present report did not affect body weight of dams (Table 1). Similarly, the number of pups...
Vascular Contractility Determinations

First, the vascular responses mediated by the NO pathway were evaluated in phenylephrine-precontracted aortic rings. The responses to L-NAME (10^{-7} - 3 \times 10^{-4} M) were used to assess the tone-related release of NO. The data also showed that the Pb regimen employed did not influence the weight of pups either at birth or at weaning (Table 1). Moreover, Pb-exposed pups presented increased Pb levels in the blood (µg/dl: control < 3.0, Pb 58.7 ± 6.5*; *p < 0.05, n = 7–10) as well as increased arterial blood pressure compared with the control group (mmHg: control 111.4 ± 2.3, Pb 135.5 ± 2.4*; *p < 0.05, n = 7–10). This result is comparable to those obtained under other dietetic regimens (Devoto et al., 2001; Lasley, 1992; Widzowski et al., 1994).

Vascular Contractility Determinations

Tone-related release of NO. First, the vascular responses mediated by the NO pathway were evaluated in phenylephrine-precontracted aortic rings. The responses to L-NAME (10^{-7} - 3 \times 10^{-4} M) were used to assess the tone-related release of NO. Perinatal Pb exposure increased the tone-related NO production in aortic tissues, that is, endothelium and smooth muscle, as observed from the higher contraction induced by increasing concentrations of L-NAME in intact and denuded aortas from Pb-exposed compared with control rats (Fig. 1 and Table 2). Moreover, the Pb-induced increase in tone-related NO production was higher in intact aortas, because the magnitude of the increase in the maximal response to L-NAME induced by Pb exposure was higher in intact than in denuded aortas (Fig. 1 and Table 2). Finally, Pb exposure did not alter the sensitivity to L-NAME of aortas with or without endothelium (Fig. 1 and Table 3).

**Stimulated release of NO.** Furthermore, the relaxant responses evoked by ACh (10^{-10} - 10^{-8} M) that trigger the release of NO from endothelial cells were investigated in phenylephrine-precontracted intact aortic rings in the absence and presence of L-NAME.

**TABLE 1**

| Body Weights of Dams and Pups Exposed or Not to Lead during Pregnancy and Lactation |
|---------------------------------|-----------------|-----------------|
| **Body weight (g)**            | **Pregnancy**   | **Lactation**   |
| 0 day                          | 21 day          | 1 day           | 21 day          |
| Control                        | 272.2 ± 6.2     | 378.8 ± 9.04    | 304.6 ± 7.0     | 276.9 ± 5.6     |
| Dams Lead (1000 ppm)           | 276.3 ± 8.2     | 367.9 ± 10.5    | 291.4 ± 8.9     | 289.8 ± 8.4     |
| Control                        | —                | —               | 6.1 ± 0.1       | 39.0 ± 0.5      |
| Pups Lead (1000 ppm)           | —                | —               | 6.0 ± 0.3       | 40.5 ± 0.8      |

*Note. Values represent means ± SE. Number of animals = 20. p > 0.05 not significant.*

In the absence of the inhibitor, ACh evoked a concentration-dependent relaxation in endothelium-intact rings that was similar between control and Pb-exposed rats (Fig. 2A and Tables 2 and 3).

Inhibition of NO synthesis by the low concentration of L-NAME (i.e., 3 \times 10^{-7} M) had no effect on the maximum response of the aortic rings from both control and Pb-exposed rats, but significantly increased the EC50 value in aortic rings from Pb-exposed rats (Fig. 2B and Tables 2 and 3). Moreover, incubation with the high concentration of L-NAME (i.e., 3 \times 10^{-5} M) completely abolished the relaxant responses evoked by ACh in aortic rings of Pb-exposed rats, whereas about 10% of the ACh response remained in aortic rings of control rats (Fig. 2C and Tables 2 and 3).

**Response to exogenous NO donor.** Endothelium-independent relaxant responses elicited by the exogenous NO donor SNP (10^{-13} - 10^{-9} M) cumulatively added to phenylephrine-precontracted intact and denuded aortic rings were similar in control and Pb-exposed rats (Fig. 3 and Tables 2 and 3).

**Cyclooxygenase pathway in aortic tissues.** In a further set of experiments, alterations in cyclooxygenase metabolites of aortic tissues were evaluated through the relaxant responses elicited by ACh in phenylephrine-precontracted intact aortic rings incubated with indomethacin (10^{-5} M) (Fig. 2D and Tables 2 and 3).
TABLE 2

Maximal Responses in the Absence or Presence of l-NAME or Indomethacin, Obtained in Aortas with and without Endothelium Isolated from Weaned Rats, Exposed or Not to Lead during Pregnancy and Lactation

<table>
<thead>
<tr>
<th></th>
<th>Maximum response (%)</th>
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<tbody>
<tr>
<td></td>
<td>With endothelium</td>
<td>Without endothelium</td>
</tr>
<tr>
<td>ACh</td>
<td>96.94 ± 1.99</td>
<td>96.57 ± 1.93</td>
</tr>
<tr>
<td>ACh/l-NAME (3 × 10⁻³ M)</td>
<td>85.38 ± 4.86</td>
<td>81.00 ± 2.88</td>
</tr>
<tr>
<td>ACh/l-NAME (3 × 10⁻⁴ M)</td>
<td>7.20 ± 1.59</td>
<td>0.01 ± 0.01*</td>
</tr>
<tr>
<td>ACh/indomethacin (10⁻⁵ M)*</td>
<td>87.75 ± 4.04</td>
<td>89.13 ± 3.13</td>
</tr>
<tr>
<td>SNP*</td>
<td>99.24 ± 1.59</td>
<td>99.80 ± 0.20</td>
</tr>
<tr>
<td>l-NAME*</td>
<td>194.30 ± 23.70</td>
<td>154.80 ± 18.30</td>
</tr>
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Note. Values are mean ± SE. *p < 0.05 significant relative to the respective control group. The endothelium removal abolished the relaxation to ACh.
Animal number range: 7–9.

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TABLE 3

EC50 Values in the Absence or Presence of l-NAME or Indomethacin, Obtained in Aortas with and without Endothelium Isolated from Weaned Rats, Exposed or Not to Lead during Pregnancy and Lactation

<table>
<thead>
<tr>
<th></th>
<th>EC50 (M)*</th>
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<tbody>
<tr>
<td></td>
<td>With endothelium</td>
<td>Without endothelium</td>
</tr>
<tr>
<td>ACh</td>
<td>1.76 × 10⁻⁷</td>
<td>5.17 × 10⁻⁷</td>
</tr>
<tr>
<td>ACh/l-NAME (3 × 10⁻⁷ M)</td>
<td>(0.44–6.98)</td>
<td>(2.22–12.05)</td>
</tr>
<tr>
<td>ACh/l-NAME (3 × 10⁻⁵ M)</td>
<td>1.32 × 10⁻⁷</td>
<td>8.88 × 10⁻⁷</td>
</tr>
<tr>
<td>ACh/indomethacin (10⁻⁵ M)*</td>
<td>(0.33–5.18)</td>
<td>(3.56–16.69)</td>
</tr>
<tr>
<td>SNP*</td>
<td>1.94 × 10⁻⁵</td>
<td>—</td>
</tr>
<tr>
<td>l-NAME*</td>
<td>0.47–8.06</td>
<td>—</td>
</tr>
<tr>
<td>Acetylcholine/indomethacin (10⁻⁵ M)</td>
<td>7.95 × 10⁻⁷</td>
<td>0.97 × 10⁻⁷</td>
</tr>
</tbody>
</table>

Note. *p < 0.05 significant relative to the respective control group. *p < 0.05 significant relative to the respective intoxicated rat in the absence of inhibitor. The endothelium removal abolished the relaxation to ACh. Animal number range: 7–9.
EC50: concentration producing half-maximum response. Values represent means with 95% confidence intervals.

DISCUSSION

The presence of the cyclooxygenase inhibitor, indomethacin, had no significant effect on the EC50 and maximum response of the intact aortic rings of the control rats (Figs. 2A and 2D and Tables 2 and 3). But in the aortic rings of Pb-exposed rats, indomethacin incubation caused significant decrease in EC50 with no significant effect on maximum response (Figs. 2A and 2D and Tables 2 and 3). Also, there was a significant difference between the EC50 in the aortic rings of the Pb-exposed rats incubated with indomethacin, compared with respective controls (Fig. 2D and Table 3).

Public health authorities use high levels to define blood lead levels of concerning in nonpregnant females (40 µg/dl, adult reference value in many countries).

In humans, the Center for Disease Control and Prevention’s (CDC’s) Adult Blood Lead Epidemiology and Surveillance program—United States, 2004 reported that 0.08 per 100,000 occupationally exposed females aged 16–44 years had blood lead levels ≥ 40 µg/dl (CDC, 2007). If a policy of universal screening were employed, the number of reported cases would be substantially higher.

We must recognize that a significant proportion of non-pregnant females with blood lead levels ≥ 40 µg/dl will become pregnant and potentially expose their infants to a risk of adverse health effects from lead. Maternal and fetal blood lead levels are nearly identical because lead crosses the placenta unencumbered. This has provoked concern about elevated blood lead levels among all females of childbearing age because a great proportion of pregnancies are unplanned. Finally, pregnant women without symptoms frequently remain in contact with the source of exposure during pregnancy and lactation, especially in developing countries.

The severity of effects and the extent to which the cardiovascular system is affected by Pb appear to be influenced most directly by Pb concentration and the duration of Pb exposure in addition to factors including route of exposure, individual life phase, temperature, and dietary calcium intake level (Kopp et al., 1988). In the present work, we investigated the vascular alterations in pups exposed to Pb during pregnancy and lactation with blood levels ≥ 40 µg/dl. The Pb exposure regimen employed in the present study caused no effect on the weight of dams and pups at birth and weaning or on the number of pups per litter. All animals appeared healthy and none of them showed signs of toxicity.
However, an increase in SBP was observed confirming previous reports showing a positive association between blood lead levels and arterial hypertension (Malvezzi et al., 2001). Behavioral and neurochemical alterations have also been reported in rats presenting similar blood lead levels (Deng and Poretz, 2001; Devoto et al., 2001).

Arterial hypertension can be associated with impaired response to ACh that is frequently related to an alteration in the NO pathway (Gao et al., 2007; Kagota et al., 2007). However, the present study indicates that ACh-induced endothelium-dependent relaxations in the aorta were unaffected by perinatal exposure to Pb. Despite this absence of effect, additional experiments were performed to determine whether the NO component of the endothelium-dependent relaxation was altered.

There is agreement in the literature that the contraction and inhibition of endothelium-dependent relaxation effects of the arginine analogs reflect inhibition of “basal” or “stimulated” activities of constitutive NOS. In order to investigate the hypothesis that plumbism could be associated with abnormality in the NO pathway, the vasoconstrictor response to L-NAME was first used to assess the tone-related release of NO. Furthermore, the relaxation evoked by ACh, which triggers the release of NO from the endothelial cell, was investigated in the presence of two different concentrations of L-NAME, $3 \times 10^{-7}$ and $3 \times 10^{-4}$M. The same strategies were used to investigate tone-related as well as stimulated release of NO after gonadectomy in rats (Cignarella et al., 2000).

The procedure reported herein showed a basal-related release of NO in both endothelium and smooth muscle of weaned rats exposed or not to Pb during pregnancy and lactation because, independently of endothelial integrity, L-NAME induced contraction in aortas from control and Pb-exposed rats. Moreover, perinatal exposure to Pb increased the tone-related

![FIG. 2. Concentration–response curves for ACh obtained in aortic rings with endothelium precontracted with phenylephrine at concentration that induced 60–80% of the maximum effect, isolated from rats exposed (triangles) or not (circles) to lead during pregnancy and lactation, in the absence (A) or presence of the inhibitors: L-NAME ($3 \times 10^{-7}$ or $3 \times 10^{-4}$M) (B and C) or indomethacin ($10^{-5}$M) (D). Loss of relaxant response occurred after endothelium removal. Data are expressed as percentage of the response elicited by phenylephrine. Values represent means ± SE. Animal number range: 7–9. *p < 0.05 significant relative to control.](https://example.com/fig2.png)
As reported above, perinatal Pb exposure did not alter the endothelium-dependent vasodilation due to ACh. However, after incubation with a low concentration of L-NAME, a specific inhibitor of NO from L-arginine, Pb-exposed rats demonstrate more depression of relaxation responses to ACh than aortic rings from control rats. Similar results were reported in aortic rings from Wistar-Kyoto and stroke-prone spontaneously hypertensive rats (Lee and Webb, 1992). These data suggest that Pb-exposed rats have altered metabolism or mobilization, or both, of L-arginine and therefore altered production of NO compared with nonintoxicated rats. The data also support the possibility that the control rats may present an alternative pathway for NO production that is not present in the intoxicated rats, because a very high concentration of L-NAME failed to completely inhibit ACh-induced relaxation in control aortic rings in contrast to those of Pb-exposed rats in which relaxation to ACh was completely inhibited.

From our data, we cannot exclude the possibility that L-NAME might have greater access to NOS in aortic rings of Pb-exposed rats than those of control rats. However, similar EC50 values for L-NAME–induced contraction in aortas from intoxicated and nonintoxicated rats suggest that the access is probably the same. Finally, similar responses to SNP, which induces relaxation independently from the endothelial NO release, would suggest that NO-mediated signal transduction in the vascular smooth muscle is equal between animal groups. Differences in responses to ACh-mediated relaxations, however, may be secondary to differences in receptor-mediated signal transduction and the increased basal release of NO in aortic tissues of intoxicated rats strongly supports this hypothesis. Differences in the L-arginine: NO pathway between intoxicated and control rats may be an important factor contributing to altered vascular reactivity in plumbism-induced hypertension.

Several studies have also shown that endothelial cell function alterations are involved in a variety of hypertensive states, a phenomenon which is probably not related to the decreased release and/or production of endothelium-derived relaxing factors (EDRFs) but to a simultaneous release of endothelium-derived constricting factors (EDCFs), for example, products of the cyclooxygenase pathway (Cordellini, 1999).

Although the production ratio of cyclooxygenase metabolites by normal cells appears to favor vasodilator substances, it is possible that this ratio may differ in response to pathological states, for example, hypertensive states (Cordellini et al., 1990). There is agreement in the literature that the use of specific inhibitors of cyclooxygenase products can clarify the involvement of cyclooxygenase-dependent dilating and/or constricting substances released from endothelial cells. In order to investigate the hypothesis that plumbism could be associated with abnormality in the synthesis of endothelium-dependent vasodilator and/or vasoconstrictor products of the cyclooxygenase pathway, the reactivity to ACh of intact aorta...
pretreated with indomethacin was studied. The presence of indomethacin determined an increase in the sensitivity to ACh in aortas from intoxicated rats without changing the vasodilation responses to this agent in control rats. These data suggest that ACh-induced vasodilatation in Pb-exposed rats, but not in control rats, is partially modulated by vasoconstrictor cyclooxygenase products whose chemical identity needs further investigation. Despite the release of cyclooxygenase metabolites, ACh-induced aorta vasodilatation in the absence of indomethacin was similar between control and intoxicated rats. In this circumstance, the increased basal NO release after Pb exposure seems to blunt the probable contribution of vasoconstrictor cyclooxygenase products in limiting ACh-induced vasodilatation in plumbism. These data corroborate previous findings from the literature showing EDCF release, sensitive to cyclooxygenase inhibitor, in macro- and microvessels of hypertensive rats (Cordellini, 1999; Cordellini et al., 1988).

Altogether, the data reported herein suggest a simultaneous release of EDRF-NO and EDCF, probably a product of arachidonic acid metabolism through the cyclooxygenase pathway, in aorta of weaned rats exposed to Pb during pregnancy and lactation. Besides the difference in sensitivity, between intoxicated and control rats in response to the inhibitory effects of l-NAME, to ACh-induced relaxation in aortic rings—the release of this constricting substance may be viewed as a mechanism involved in the etiology and/or maintenance of the hypertensive state after Pb intoxication. Moreover, the increased basal release of NO might be considered a mechanism to counteract the vasoconstrictor production and the hypertensive state developed as a consequence of perinatal Pb exposure. This raises concern about the higher cardiovascular vulnerability of Pb-exposed individuals in the presence of associated pathologies that impair NO-system activity.

These findings may aid in understanding of the contribution of NO and cyclooxygenase products to the etiology and/or maintenance of the hypertensive state in Pb intoxication and could ultimately lead to therapeutic advantages against cardiovascular diseases in plumbism.

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