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Lead effects on non-adrenergic non-cholinergic relaxations in the rat gastric fundus

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Abstract

The effect of lead exposure on non-adrenergic non-cholinergic (NANC) relaxations in rat gastric fundus was evaluated in this work. Wistar rats were divided into four groups: The control group received tap water and the three other received 0.008% of lead acetate in their drinking water for 15, 30 and 120 days. NANC relaxations induced by electrical field stimulation (0.5–8 Hz, 1 ms, 60 V) of gastric fundus strips was inhibited in all groups treated with lead. The strips from groups, control and 120 days of lead treatment (LEAD 120), were incubated with L-NOARG (100 μM). The presence of this blocker did not produce any additional inhibition. Sodium nitroprusside (10^{-10}–10^{-6} M) and 8-Br-GMPc (3×10^{-8}–3×10^{-4} M) produced dose-dependent relaxations in strips of both groups control and LEAD 120, however, in the LEAD 120, the potencies were significantly reduced from 7.32 ± 0.05 to 6.40 ± 0.09 ($n = 5$) and 4.26 ± 0.06 to 3.69 ± 0.05 ($n = 5$), respectively. Our data suggest that the chronic exposure to lead inhibits NANC relaxations probably by modulating NO release from NANC nerves and/or by interacting with intracellular transducer mechanisms in rat gastric fundus.

Keywords: Lead; NANC relaxations; Nitric oxide; Rat gastric fundus

1. Introduction

Lead is a potent environmental toxicant frequently found in air, drinking water, soil, wall fixtures, dusts, lead-based paints and industrial byproducts (Singh, 1993). Chronic exposure to lead has shown to cause cerebral neuropathy, liver damage, behavioral abnormalities (Needleman et al., 1979; Annest et al., 1983; Moore et al., 1986) and hypertension (Khall-Manesh et al., 1994). Part of neurotoxic effects of lead in the central nervous system is due to a decrease in the release of gamma-aminobutyric acid (GABA), acetylcholine and noradrenaline (Silbergeld et al., 1980; Ramasay et al., 1980; Pickett and Bornteins, 1984; Moore et al., 1986). Other studies report that lead may exert its toxicity through the modulation of nitric oxide (NO) production (Blazka et al., 1994; Tian and Lawrence, 1995). Nevertheless, it is difficult to study exactly how lead affects synaptic transmission in the central nervous system. For this reason, in this study we used an in vitro peripheral nervous system preparations.

Non-adrenergic non-cholinergic (NANC) fibers are the major inhibitory neurons of the myenteric plexus of the gastric fundus and NO, its main neurotransmitter (Smits and Lefebvre, 1995). In these nerves, NO and L-citruline are formed by Ca^{2+}-calmodulin dependent NO-synthase (NOS) from L-arginine (Bredt et al., 1992).

Abbreviations: EFS, electrical field stimulation; GABA, gamma-aminobutyric acid; L-NOARG, L-nitroarginine; NANC, non-adrenergic non-cholinergic; NO, nitric oxide; NOS, nitric oxide synthase; SNP, sodium nitroprusside.

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The synthesis of NO can be inhibited by substitutive L-arginine analogues, such as L-nitroarginine (L-NOARG). NO acts through activation of soluble guanylate cyclase, increasing the intracellular cGMP concentration and promoting relaxation (Moncada et al., 1991). Therefore, the aim of this work was to evaluate the effects of chronic lead exposure on non-adrenergic non-cholinergic (NANC) relaxations in rat gastric fundus.

2. Material and methods

2.1. Exposure of rats to lead

Male Wistar rats (*Rattus norvegicus*) weighing 100–200 g were distributed into four groups (one control and three experimental) and housed (four animals per cage) under conditions of controlled temperature (25 ± 1 °C) and lighting (lights on: 6–18 h). Animals had access to food and water ad libtum. The experimental groups received lead acetate (0.008%; w/v) through drinking water for 15, 30 and 120 days, while the control group received tap water.

2.2. Tissue preparation

All animals were killed by stunning and exsanguination, and longitudinal strips (2–2.5 cm) of gastric fundus were mounted in organ baths filled with 10 mL of Krebs solution containing atropine (1 μM) and guanethidine (3 μM) (composition in mM: NaCl 118.5, KCl 4.8, $CaCl_2$ 1.9, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 10.1). The strips were placed under ±1 g of resting tension for a stabilization period of 60 min. During this time, the solution was changed at 15 min intervals to prevent the accumulation of metabolites. The solution was maintained at 37 °C and gassed with a mixture of 95% O_2/5% CO_2. The strips were connected to a force transducer coupled to an amplifier-recorder (GOLD, USA) for isometric tension recording.

Electrical field stimulation (EFS) was applied through two platinum electrodes placed below and above the strips.

2.3. Experimental protocols

After the stabilization period, all strips were first contracted with $PGF_{2\alpha}$ (0.1 μM) and then a stable sustained contraction, EFS (0.5–8 Hz, 1 ms, 60 V) was produced in order to get frequency–response curves. In order to verify a possible damage to the tissue by lead treatment, papaverine (1 μM) was added to the bath in the end of each experimental protocol.

For the group of 120 days of lead treatment (LEAD 120), the strips were incubated for 30 min with 100 μM of L-NOARG, an inhibitor of the NOS (Moncada et al., 1991), before the $PGF_{2\alpha}$ contraction. Then, a frequency–response curve to EFS was obtained. In another set of experiments, the $PGF_{2\alpha}$-contracted strips of both groups, control and LEAD 120, were relaxed cumulatively with sodium nitroprusside (SNP) (10^{-10}–10^{-6} M), a NO donor, and 8-Br-GMPc (3×10^{-8}–3×10^{-4} M), a stable cGMP analog.

2.4. Drugs

The drugs used were: Atropine sulphate, guanethidine sulphate, L-nitroarginine (L-NOARG), sodium nitroprusside, 8-bromo-cGMP, $PGF_{2\alpha}$ and papaverine hydrochloride (all from SIGMA). All drugs were dissolved in distilled water and prepared freshly before each experiment.

2.5. Statistics

All values were expressed as the mean ± SEM. Differences between means were assessed by Student's unpaired *t* test. Values of $p < 0.05$ were accepted as statistically significant. The pD_2 values were obtained by non-linear regression using the Graph Pad Prism statistics software version 3.02.

3. Results

3.1. NANC relaxations induced by EFS

As shown in Fig. 1, EFS produced frequency-dependent relaxations in rat gastric fundus of all groups, however in the lead-treated groups (15, 30 and 120 days of treatment), the relaxations were significantly attenuated when compared with control. Lead exposure time affected significantly the magnitude of NANC relaxations. The maximal response were reduced from 80 ± 3% (control group) to 66 ± 2%, 55 ± 4% and 19 ± 2% (15, 30 and 120 days of treatment, respectively) ($p < 0.05$; $n = 5$). Papaverine (1 μM) was able to relax all preparations when added in the end of experimental protocols (data not shown).

3.2. Effect of L-NOARG on NANC relaxations induced by EFS

As shown in Fig. 2, L-NOARG (100 μM) inhibited the NANC relaxations induced by EFS only in the control group. The maximal response was significantly reduced from 80 ± 3% to 18 ± 0.6% ($p < 0.05$, $n = 5$). Interestingly, these relaxations were not different from those obtained from the LEAD 120 group, demonstrating that L-NOARG was not capable of producing additional inhibition.