INTERACTION OF L-ARGININE/NITRIC OXIDE SYSTEM WITH LEAD ACETATE ON SECRETION OF AMYLASE FROM ISOLATED RAT PAROTID GLANDS

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ABSTRACT

In the present study the effects of lead acetate and/or L-Arginine as a nitric oxide precursor and L-NAME as a nitric oxide synthase inhibitor on the amylase secretion of rat parotid gland lobules were investigated. Lead acetate in doses of 3, 30 and 300 μM significantly ($p<0.01$) caused a dose-dependent reduction in isoproterenol-stimulated or non-stimulated amylase secretion. When secretion of saliva was not stimulated by beta-adrenergic agonist, L-Arginine (100 μM) significantly ($p<0.01$) reduced amylase output. L-NAME (100 μM) alone had no significant effect on amylase output but when used with lead acetate prevented ($p<0.01$) from lead-induced reduction of amylase output. Both L-NAME (100 μM) and L-Arginine (100 μM) when used alone reduced isoproterenol-stimulated amylase output. Concurrent administration of lead acetate (300 μM) with either L-Arginine (100 μM) or L-NAME (100 μM) showed a marked positive interaction in reducing the isoproterenol-stimulated secretion of amylase. These findings suggest that nitric oxide plays a role in secretion of amylase from parotid. Different affinity of lead acetate to interact with different nitric oxide synthases might be a reason for different effects on parotid amylase secretion observed in the presence or absence of secretion stimulant.

Key words: Lead, Parotid, Amylase, Nitric oxide, L-NAME

INTRODUCTION

Salivary amylase has been considered to be of significance for dental health because of its intraoral actions. Amylase in the salivary glands is mainly released through the intracellular cAMP pathway by beta-adrenoceptor stimulation (1,2).

The extensive use of lead compounds in various branches of industry and general life makes intoxication with this metal one of the most serious toxicological problems (3,4). Toxic effects of lead on many organs have been found in animals and humans but little is known on its effects on parotid secretion of amylase. Secretion of lead into submandibular and parotid saliva of rat (5,6) and its accumulation in salivary gland have been found (7,8). Alteration of rat submandibular secretory function and degenerative ultrastructural changes of gland have been also reported (5,7).

On the other hand it has been found that nitric oxide also plays a potential role in modifying oral mucosal diseases as a physiopathological regulator (9). Nitric oxide is present in saliva and its possible sources might be the periacinar neural baskets, periductal fibers and/or ductal epithelial cells (10). Marked specific activity of nitric oxide synthase was detected in submandibular and parotid glands of rats (11,12). It has been suggested that several elements within the salivary gland, such as ganglion cells, periductal, periacinar, perivascular nerves synthesize and may use nitric oxide as a putative neurotransmitter.

It has been proposed that environmental toxicant lead may exert their toxicity through modulation of nitric oxide production (13). There is evidence that lead can efficiently block nitric oxide production in vitro *(14)* and in brain (15). Our recent study showed that nitric oxide synthase inhibitor can

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potentiate lead-induced effects in rat submandibular gland function (16) and the similar thing is reported for cadmium (17).

Since amylase is an important enzyme that is mainly secreted from parotid, we were interested to examine whether lead acetate modify the release of amylase in the presence of nitric oxide precursor or synthase inhibitor in a rat parotid gland in vitro.

MATERIALS AND METHODS

Chemicals:

Lead acetate was obtained from Merck (Germany), Isoproternol sulphate, N^{ω}-nitro-L-Arginine methyl ester (L-NAME), L-Arginine and all other chemicals were purchased from Sigma Chemical Co. (UK).

Methods:

Forty-eight male Sprague-Dawley rats weighting 200-300 g were kept in an environment at 21-24°C with a light/dark cycle of 14/10hr starting at 6:00 a.m. and allowed to become acclimatized to standard laboratory conditions for at least seven days. Tests were performed after they had been starved for 16 hours, starting at 09:00-10:00 hours, to rule out the effect of circadian rhythm on the glands secretions. Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/Kg). They were then secured in a supine position with tape. Then left and right parotid glands were dissected free of connective tissues, removed and transferred to ice cold Krebs-Ringer-Tris (KRT) solution, containing (in mM) NaCl, 120; KCl, 4.8; KH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 3.0; Tris HCl buffer, 16 (pH 7.4); and glucose, 10 (18). Connective tissues, fat and lymph gland were then removed and the ventromedial portion of the parotid glands of each rat (19) was cut in two pieces (20).

One piece of parotid gland from each rat, with a total weight of about 100 mg, was cut into pieces and equilibrated, separately for each rat, in an organ bath containing 10 ml of KRT solution in the presence of atropine sulphate (20 μM) and phenoxybenzamine HCl (10 μM) (19-21) for 20 minutes at 37°C while shaking continuously at 90 cycle/min. During the experiments, the KRT solution was oxygenated. The test drugs (Lead acetate, L-NAME and L-Arginine) were administered after the equilibration period and 10 μM isoproterenol (21,22) was added 80 min after incubation with the test drugs and incubated for another 80 minutes period.[19] In the controls, equivolume amount of the solvent (deionized water) was administered. Samples of 20 μl. were obtained at definite time intervals (0,20,40,60,80,90,100,110,120 and 160 minutes) and then determined for rate of amylase output.

Amylase determination:

Amylase activity was measured as described previously (23). The cumulative and absolute amylase output rates were expressed as rate of amylase secretion per mg wet weight of gland multiplied by 100 per min. For determining the absolute rate of amylase secretion, each one was subtracted from its former one.

Statistical analysis:

Values are reported as mean±SE. Statistical significance between groups was computed using analysis of variance and Newman-Keul's tests. P values greater than 0.05 were considered insignificant.

RESULTS

Lead acetate reduced amylase output in a dose dependent manner when compared to control (Figure 1A). The cumulative amylase output (%) (U/mg) after 80 minutes was 187.75±9.3 (for control), 164.25±8.29 (for 3 μM lead acetate, $p<0.01$), 150.2±9.00 (for 30 μM lead acetate, $p<0.01$), and 116.6±6.78 (for 300 μM lead acetate, $p<0.01$).

Figure 1B shows that the absolute amylase output reached maximum level at the 20th min after adding of isoproterenol, at which all doses of lead acetate 3, 30, and 300 μM caused a significant reduction ($p<0.01$). The values were 32±2.5 (for control), 24.5±2.1 (for 3 μM lead acetate), 19.8±1.8 (for 30 μM lead acetate) and 15±1.4 (for 300 μM lead acetate).

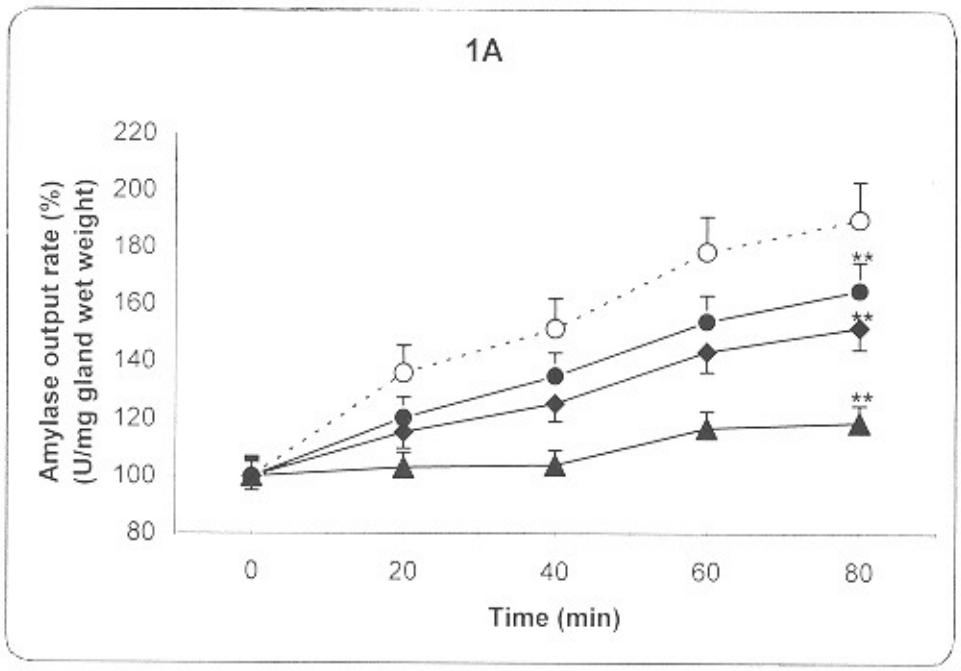

Fig. 1A. Effects of various doses of lead acetate on cumulative amylase secretion from rat parotid glands. Each point is the mean of 6 observations and is represented as mean±SE. Control (○), lead acetate in doses of 3 μM (●), 30 μM (◆), 300 μM (▲). **Difference from control is significant ($p<0.01$).

Figure 2A shows the effect of L-NAME (100 μM) and L-Arginine (100 μM) alone and in concomitant administration with 300 μM lead acetate on amylase output. L-NAME alone had no significant effect on amylase output when compared to control but when used with lead acetate partly prevented lead-induced reduction of amylase output significantly ($p<0.01$) at 80 min but did not reach control levels. L-Arginine alone significantly ($p<0.01$) reduced amylase output while in concomitant use with lead acetate did not affect lead-induced reduction of amylase output at 80 min.

Figure 2B represents the effects of L-NAME and L-Arginine alone and in concomitant administration with 300 μM lead acetate on isoproterenol-stimulated amylase output. Lead acetate, L-NAME and L-Arginine when used alone reduced amylase output. Concurrent use of L-NAME with lead acetate and L-Arginine with lead acetate reduced amylase output and showed potentiation effect in comparison to that of lead acetate ($p<0.01$).

DISCUSION

The present study shows that lead acetate in different doses used (3, 30 and 300 μM) decreases parotid amylase activity in a dose-dependent manner (Fig. 1A,B). Amylase release from parotid acinar cells is mainly induced by accumulation of intracellular $cAMP^{2+}$ that is under beta-adrenergic system control (2,25). Calcium is determined to be the common intracellular mediator for these regulatory processes and increased intracellular calcium concentration acts as a signal or second messenger in secretion of amylase via salivary glands (26,27).

Lead may substitute for calcium in many intracellular regulatory events (28-30). Thus significant activities in the salivary glands of animals can be completely blocked in the absence of calcium or the presence of a calmodulin inhibitor (25,27). Thus, possible replacing of calcium with lead and decreasing intracellular calcium may be reasons for observed alterations. Previously it was reported that lead acetate causes a significant reduction in stimulated flow rate, total protein and some electrolyte disturbances (5).

As well, marked changes in ultrastructural features of acinar cells of the gland, such as irregular patterns of nucleus, mitochondrial alteration with decreased DNA content were the most striking evidences for lead acetate administration in rat submandibular gland (7).

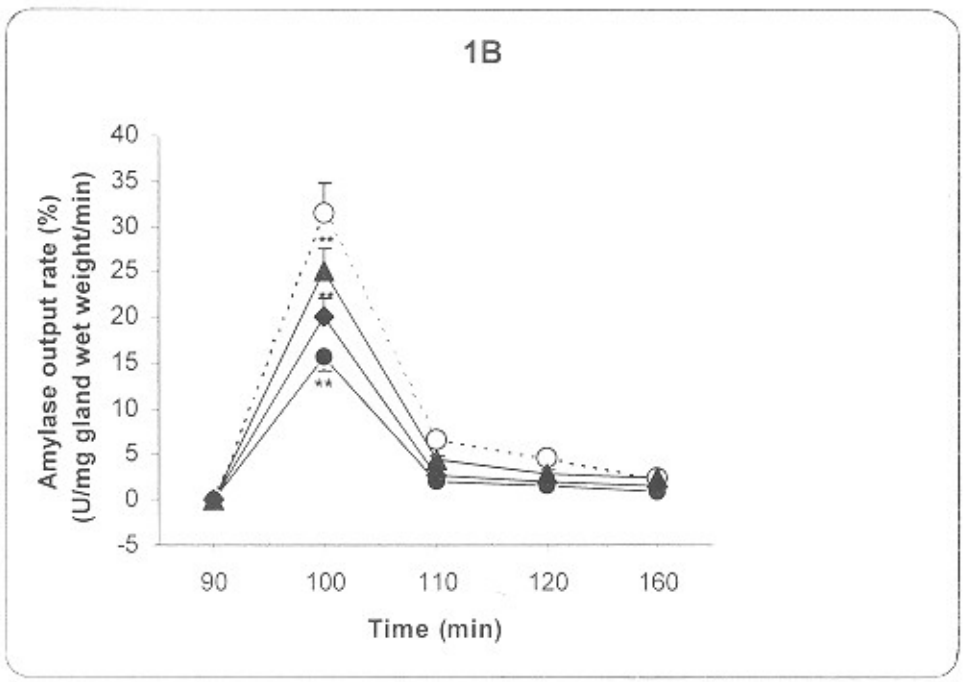

Fig. 1B. Effects of various doses of lead acetate on isoproterenol-induced (absolute) amylase secretion from rat parotid glands. Each point is the mean of 6 observations and is represented as mean±SE. Control (○), lead acetate in doses of 3 μM (●), 30 μM (◆), 300 μM (▲). **Difference from control is significant ($p<0.01$).

It is possible that lead acetate has the same properties on rat parotid gland, thus destruction of mitochondria, energy deficiency, hindered of transport and prevented synthesis of various proteins, including amylase could be predicted. The present study shows that inhibitory effects of lead acetate on salivary glands in vitro fully match with that of in vivo findings as above mentioned. It indicates that this in vitro model can be an efficient tool for investigation on salivary glands.

At the second part of the study, one of effective doses of lead acetate was selected to study its concurrent effects with nitric oxide precursor or synthase inhibitor. Our previous studies showed that lead acetate could enter and accumulate in salivary glands with high concentrations (6,7). Results show that concurrent administration of lead acetate (300 μM) and L-NAME (100 μM) partly prevents lead-induced reduction of amylase output (Fig. 2A) but when isoproterenol was used, both L-NAME (100 μM), and L-Arginine potentiated lead-induced reduction of amylase output (Fig. 2B).

Our recent in vivo study on rat submandibular gland showed that concurrent use of lead acetate with L-NAME, potentiates inhibitory effect of lead in cholinergic-stimulated secretion of protein (16), which supports the adrenergic-stimulated experiment in the present study. Additionally opposite effects of L-Arginine and L-NAME in the secretion of protein and calcium was reported that is not in accordance with the present finding. There is another report indicating that following parasympathetic stimulation of parotid secretion in anaesthetized lambs, administration of L-NAME reduces the rate of protein secretion (31). They found that output of protein in response to autonomic stimulation exhibits a greater nitric oxide dependence that partly supports the differences observed between isoproterenol-stimulated and non-stimulated amylase secretion in the present study.

As reviewed recently (32) nitric oxide is synthesized from the aminoacid L-Arginine by nitric oxide synthase. Nitric oxide is an important signaling molecule that, besides acting as a signal transducer, exerts a variety of regulatory and cytostatic functions. Two classes of nitric oxide synthase (constitutive and inducible) exist in higher animals and are conserved between species. The constitutively expressed isoforms are tightly regulated by Calcium/calmodulin and are involved mostly in regular housekeeping functions in the body.

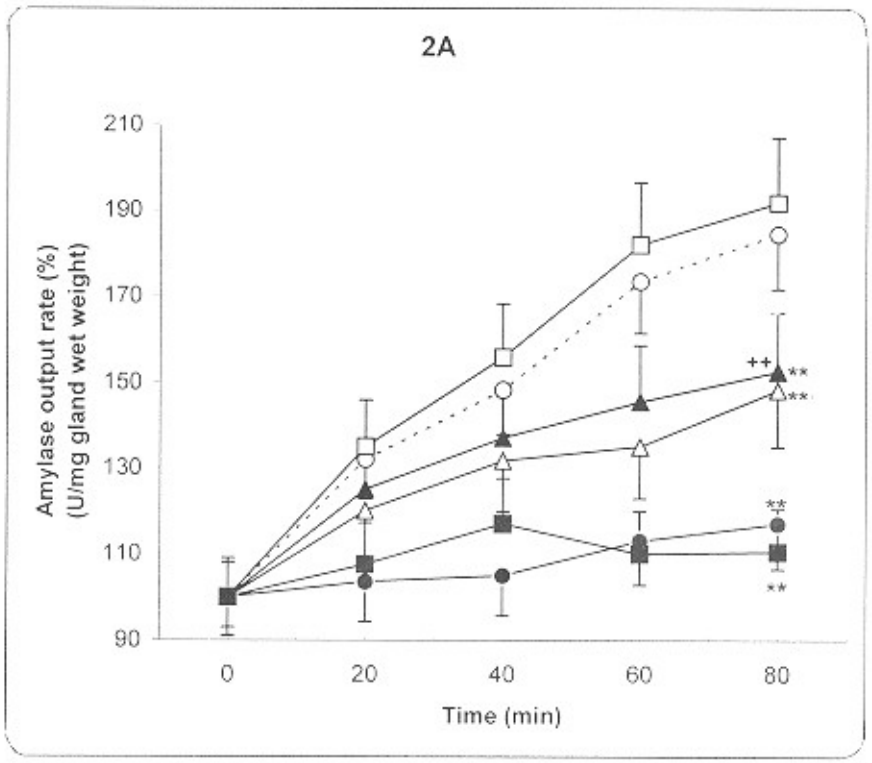

Fig. 2A: Effects of lead acetate alone and its concomitant administration with L-Arginine and L-NAME on cumulative amylase secretion from rat parotid gland. Control (○), lead acetate 300 μM (●), L-NAME 10 μM (□), L-Arginine 100 μM (△), lead acetate 300 μM + L-Arginine 100 μM (■), lead acetate 300 μM + L-NAME 100 μM (▲). Each point is the mean of 6 observations and is represented as mean±SE. **Difference from control is significant ($p<0.01$). ++Difference from lead acetate-treated group is significant ($p<0.01$).

The inducible isoform is activated transcriptionally after stimulation of various cells by proinflammatory signals and once expressed is active over longer time periods without any major short-term regulation. Nonselective inhibitors can deteriorate conditions due to concomitant inhibition of constitutive isoforms. Inhibition of the constitutive endothelial isoform results usually in deterioration of normal condition of organ. Based on a new radioligand binding experiment L-NAME has been found as a selective and potent constitutive nitric oxide synthase inhibitor (33). Constitutive isozymes do not contain bound calmodulin, but in the presence of calcium, association occurs with high affinity, resulting in enzyme activation (32,33). Interaction of heavy metal cations may be exerted on the constitutive nitric oxide synthase catalytic site(s) by direct binding to it or by interfering with the electron transfer during catalysis (14,15,34).

Considering properties of lead to interact with calcium- and calmodulin-dependent processes, its interaction with constitutive nitric oxide synthase is anticipated (35). Thus, it inhibits production of nitric oxide, which in the presence of L-NAME could make a higher degree of inhibition on isoproterenol-stimulated secretion of amylase (Fig. 2B). When L-Arginine is used, inducible nitric oxide synthase could be activated that might lead to high and cytotoxic nitric oxide levels, which in turn could disturb isoproterenol-stimulated secretion of amylase (Fig. 2A,B). It has been reported that N(G)-nitro-L-arginine (NOLA) as a non-selective nitric oxide synthase inhibitor can increase carbachol-stimulated secretion of amylase which in turn can be prevented by L-Arginine (36).

According to this report propranolol administration decreases amylase secretion but NOLA cannot modify this suppressed secretory rate that is not in accordance with our results.

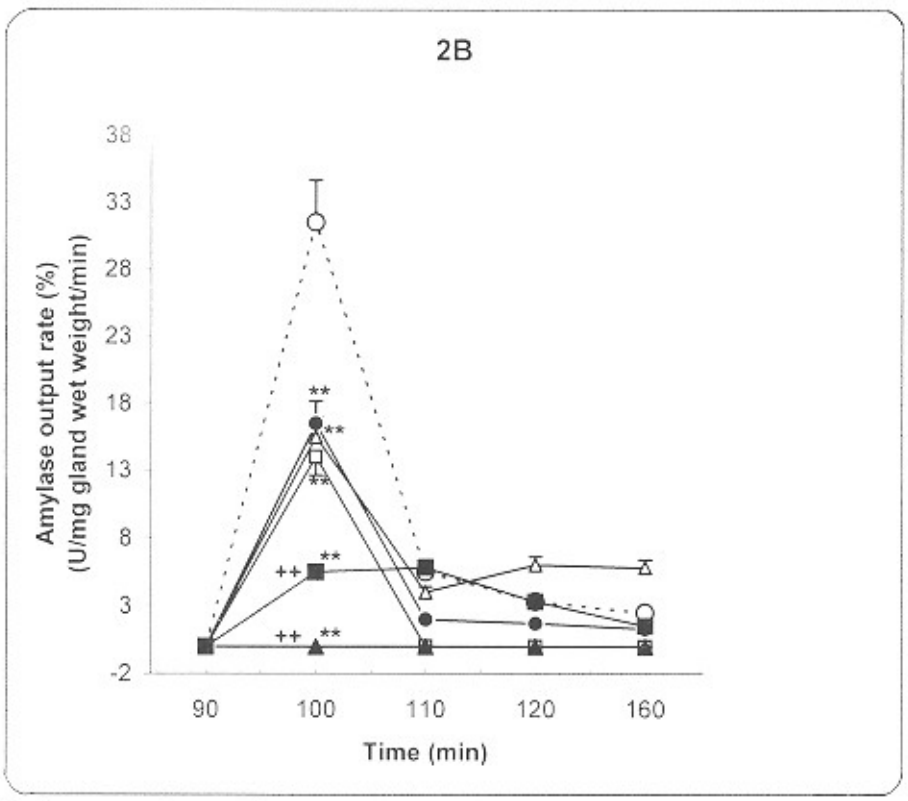

Fig. 2B: Effects of lead acetate alone and its concomitant administration with L-Arginine and L-NAME on isoproterenol-induced (absolute) amylase secretion from rat parotid gland. Control (○), lead acetate 300 μM (●), L-NAME 10 μM (□), L-Arginine 100 μM (△), lead acetate 300 μM + L-Arginine 100 μM (■), lead acetate 300 μM + L-NAME 100 μM (▲). Each point is the mean of 6 observations and is represented as mean+SE. **Difference from control is significant ($p<0.01$). ++Difference from lead acetate-treated group is significant ($p<0.01$).

It is important to mention that the inhibitor of nitric oxide synthase was different in these two experiments. We believe that since secretion of amylase in parotid cells is mainly under control of sympathetic control (18), thus inhibitory effects of L-Arginine and L-NAME on amylase secretion should be examined under sympathetic stimulation not cholinergic. When amylase secretion was not stimulated by isoproterenol, L-NAME alone had no significant effect on amylase output but prevented lead-induced reduction of amylase output (Fig 2A). This effect of L-NAME is completely in contrast with that of isoproterenol-stimulated amylase experiment. One explanation for this contrast may be that L-NAME could inhibit different isoforms of nitric oxide synthases dependent on presence of autonomic stimulator. This finding can also be a reason for discrepancies that are observed between different studies.

Based on the obtained results, interaction of lead and nitric oxide in parotid gland is confirmed that makes a support to previous findings on interaction of lead and nitric oxide in other biological systems including endothelial cells (13,37), macrophages (14), submandibular gland (16,17), Kidney (38) and central nervous system (15,35). Nevertheless the controversy on exact role of nitric oxide in many organs as well as parotid gland still exist and remains to be elucidated by further studies.

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