Jpn. J. Vet. Res. **42**(3-4) : 109-118 (1994)

THE INHIBITORY ACTION OF LEAD ON MECHANICAL RESPONSES OF THE PROVENTRICULAR SMOOTH MUSCLE IN THE CHICK

Kazufumi KUBOTA[1], Shigeo ITO[1], Toshio OHTA[1], Yoshikazu NAKAZATO[1] and Akira OHGA[2]

(Accepted for publication : Nov. 8, 1994)

ABSTRACT

The purpose of the present experiments was to examine the mechanism of the proventricular dilatation caused by lead in the isolated vagus nerve-proventricular smooth muscle preparation of the chick. Lead caused dose- and time-dependent inhibition of contractions induced by vagal stimulation, transmural stimulation and externally applied acetylcholine (ACh). Vagally evoked contraction was much more sensitive to the inhibitory action of lead than the contractile response to ACh. The lower the frequency of transmural stimulation, or the lower the concentration of ACh was applied, the greater the inhibitory action of lead on the evoked smooth muscle contraction. The results suggest that proventricular impaction occurring in lead poisoning results from the pre- and post-synaptic inhibition of the vagus nerve-smooth muscle transmission.

Keys words : Autonomic transmission, Ca^{2+} influx, chick stomach

INTRODUCTION

A variety of waterfowls has been suffering from lead poisoning which is primarily the result of ingestion of spent lead shot from the bottom of lakes and marshes. The clinical signs of lead intoxication in waterfowls are lethargy, inappetence, green liquid feces, ataxia, emaciation and so on. In addition, the proventriculus and lower oesophagus are often impacted with feed[9]. Proventricular impaction and the residue of lead shot are observed in living waterfowls by X-ray.

It has been reported that lead inhibits the release of acetylcholine (ACh) at the neuromuscular junction[5,8] and sympathetic ganglia[3] by decreasing Ca^{2+} influx required for transmitter release. Lead has also been shown to inhibit the adrenergic

[1] Department of Pharmacology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

[2] Department of Veterinary Medicine, Faculty of Dairy Science, Rakuno Gakuen University, Ebetsu 069, Japan

neurotransmission[1]. Therefore, it is possible that proventicular impaction is due to the disfunction of the autonomic nerves which innervate the proventriculus. However, there has been no experimental evidence for this possibility. The purpose of the present experiments was to examine the mechanism of the proventricular dilatation induced by lead in the isolated vagus nerve-proventricular smooth muscle preparation of the chick.

MATERIALS AND METHODS

Chicks 2–5 weeks after hatching were anesthetized with chloroform and bled to death and then the proventriculus with the vagus nerve was isolated. The contents of proventriculus were washed out with physiological salt solution after longitudinal incision. The mucous and glandular tissues were then detached with a scissor in physiological salt solution and a segment of longitudinal muscles, about 3–4 mm wide and 15–20 mm long, was taken with the vagus nerve attached[6]. The isolated vagus nerve-smooth muscle preparation was mounted in a 3 ml organ-bath at 37°C and loaded with 1 g tension.

The physiological salt solution was the following composition (mM) : NaCl 144 : KCl 5.8 ; $MgCl_2$ 1.2 ; $CaCl_2$ 2.5 ; glucose 11.1 ; 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonate (HEPES) 5. The pH was adjusted with NaOH (pH 7.4). The medium in the organ bath was bubbled with O_2 throughout the experiments.

The experiment was started 30–60 min after equilibration of the preparation. The mechanical response was recorded isotonically with a mechano-electric transducer (TD-112S Nihon Kohden). The vagus nerve was stimulated with a suction electrode which consisted of two silver wires, one rolled around the tip of a glass tube about 1.5 mm in diameter, and the other placed inside the tube. Transmural electrical stimulation was applied through two parallel, Ag-AgCl electrodes (5 × 20 mm, separated by 5 mm) placed on either side along the whole length of the smooth muscle segment. Drugs were applied to the organ bath with a micropipette and washed out by overflow. All results are expressed as the mean ± SEM.

Chemicals used were : acetylcholine chloride (Ovisot, Daiichi), atropine sulphate monohydrate, lead chloride (Wako Pure Chem) and 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonate (HEPES, Dojindo).

RESULTS

Inhibitory effects of lead on contraction induced by vagal stimulation and acetylcholine

Stimulation of the vagus nerve (0.5 Hz, 1 msec, 10 V) attached to smooth muscles isolated from the proventriculus for 6 sec produced a transient contraction which was blocked by atropine. ACh at concentrations between 0.1 and 0.3 μM (mean concentration, 0.18 ± 0.06 μM, n=7) also produced a contraction, the magnitude of which was similar to that induced by vagal stimulation at 0.5 Hz. Fig. 1 shows

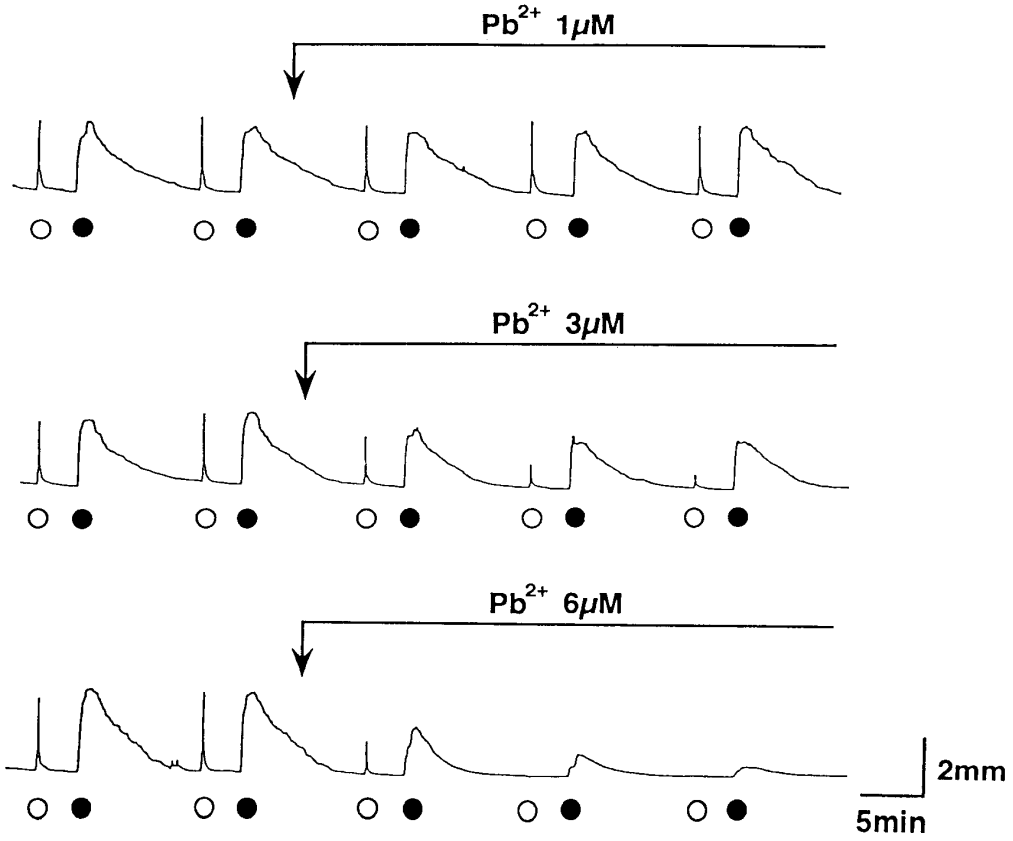

Fig. 1. The inhibitory effect of lead on contractions induced by vagal stimulation (○) and ACh (●). The vagus nerve was stimulated at 0.5 Hz, 1 msec, 10 V for 6 sec. The concentration of ACh was 0.16 μM. Horizontal bars with arrows indicate the periods of application of lead at the concentration described. Representative data are illustrated.

the effect of lead on contractions induced by vagal stimulation and ACh. Lead at a concentration of 1 μM had no effect on contraction evoked by vagal stimulation and ACh. When the concentration was increased to 3 μM, lead inhibited the contraction induced by vagal stimulation more effectively than that induced by ACh. ACh-induced contractions were also decreased by a further increase in the concentration of lead to 6 μM.

The inhibitory effect of lead on both contractions appeared to be time-dependent after treatment with lead. Therefore, the percentage of contractions obtained from the experiments with the same protocol shown in Fig. 1 were plotted against the time after treatment with lead at various concentrations (Fig. 2). Lead caused time- and concentration-dependent decreases in contractions in response to vagal stimulation and

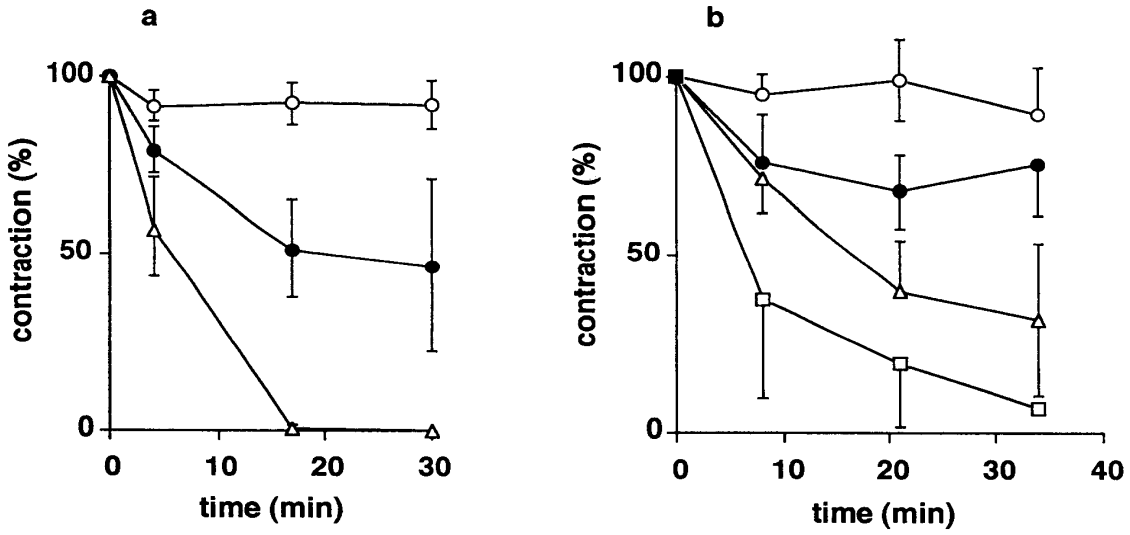

Fig. 2. Time- and dose-dependent inhibition by lead of contractions induced by vagal stimulation (a) and ACh (b). The contraction induced by vagal stimulation or ACh is expressed as a percentage of that obtained in the absence of lead. Each symbol indicates the mean value from 3 to 6 experiments in the presence of lead at concentrations of 1 μM (○), 3 μM(●), 6 μM (△) and 10 μM (□). Vertical bars represent SEM.

ACh. The contraction induced by ACh was more resistant to lead than that induced by vagal stimulation.

Effects of lead on contractions induced by transmural nerve stimulation with various frequencies

The responses to higher frequencies of vagal stimulation were easy to fatigue and those to lower frequencies of stimulation were hardly recovered from the inhibitory effect of lead. Transmural stimulation is shown to produce a cholinergic contraction in the present preparation as well as vagal stimulation[7]. Therefore, we examined the effect of lead on contractions induced by transmural electrical stimulation. Transmural stimulation at 0.5 Hz (1 msec, 10 V) for 6 sec caused a transient contraction which was much more reproducible and stable than vagally induced one. Lead at concentration of 3 μM or more caused dose- and time-dependent inhibition of contractions induced by transmural stimulation (Fig. 3). The inhibitory effect developed in a dose-dependent manner with any frequency of stimulation applied, though the rate of inhibition was much greater in the contractions induced by lower frequencies of stimulation than those by higher frequencies (Fig. 4).

Effects of lead on contractions induced by acetylcholine at various concentrations

ACh was cumulatively applied to the bathing solution to construct dose-contraction curves in the presence and absence of lead (Fig. 5). In the absence of lead, the response to ACh appeared at a concentration of 10 nM and increased with increasing concentration up to 1 mM. The magnitude of the contraction induced by ACh at 0.1 μM was comparable to those by transmural stimulation at 0.5 or 2 Hz. Lead dose-dependently inhibited the contraction induced by ACh at concentration lower than 10 μM. The lower the concentration of ACh, the greater the inhibitory effect of lead. The ED_{50} values for ACh increased with increasing the concentration of lead.

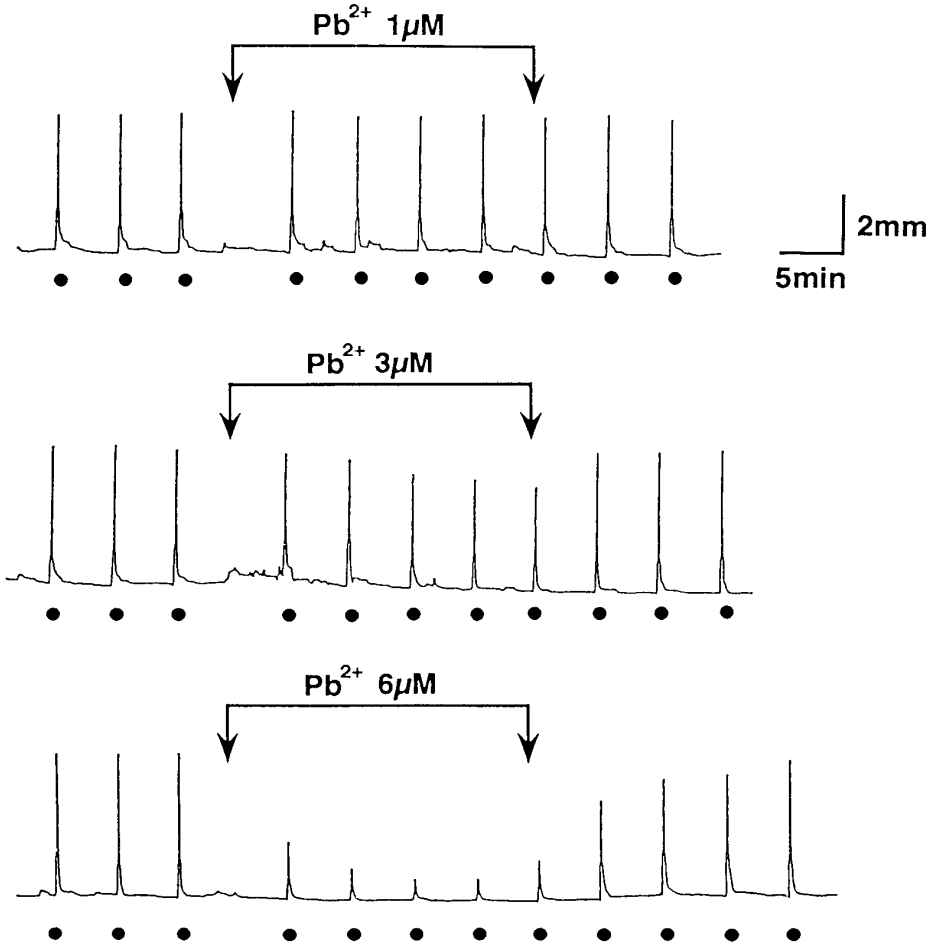

Fig. 3. The inhibitory effect of lead on the contractions induced by transmural nerve stimulation. Transmural stimulation at 0.5 Hz (●, 1 msec, 40 V) was applied for 6 sec. Horizontal bars with arrows indicate the periods of application of lead at the concentration described. Representative data are illustrated.

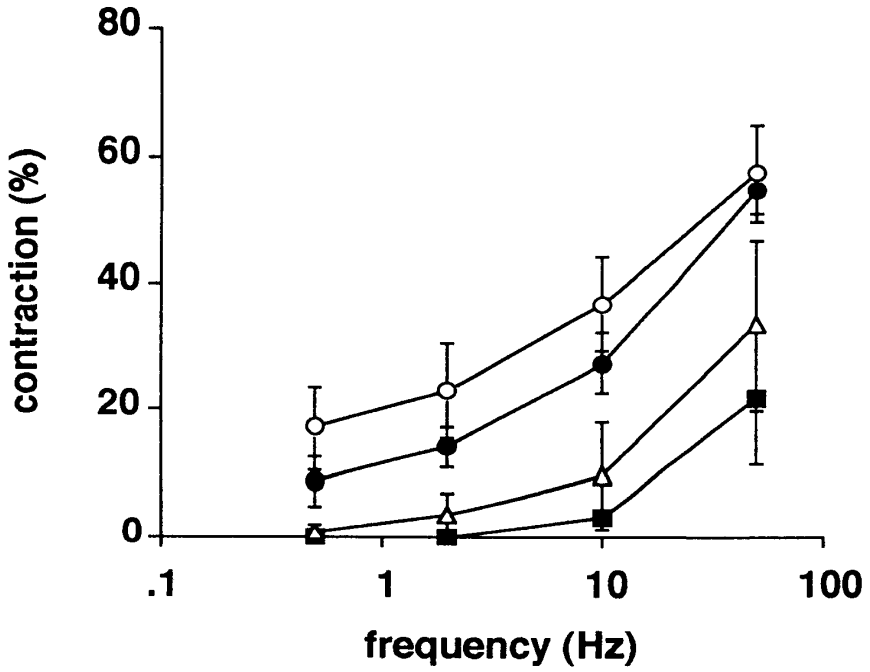

Fig. 4. Effect of lead on frequency-response curves for transmural nerve stimulation. The contraction induced by transmural stimulation is expressed as a percentage of that induced by 1 mM ACh. Symbols indicate the mean values obtained from 3 to 5 experiments in the absence of lead (○) and presence of lead at concentrations of 5 μM (●), 10 μM (△) and 20 μM (■). After control responses were obtained, the preparation was exposed to lead for about 20 min and then transmural stimulation was applied in the presence of lead. Vertical bars represent SEM.

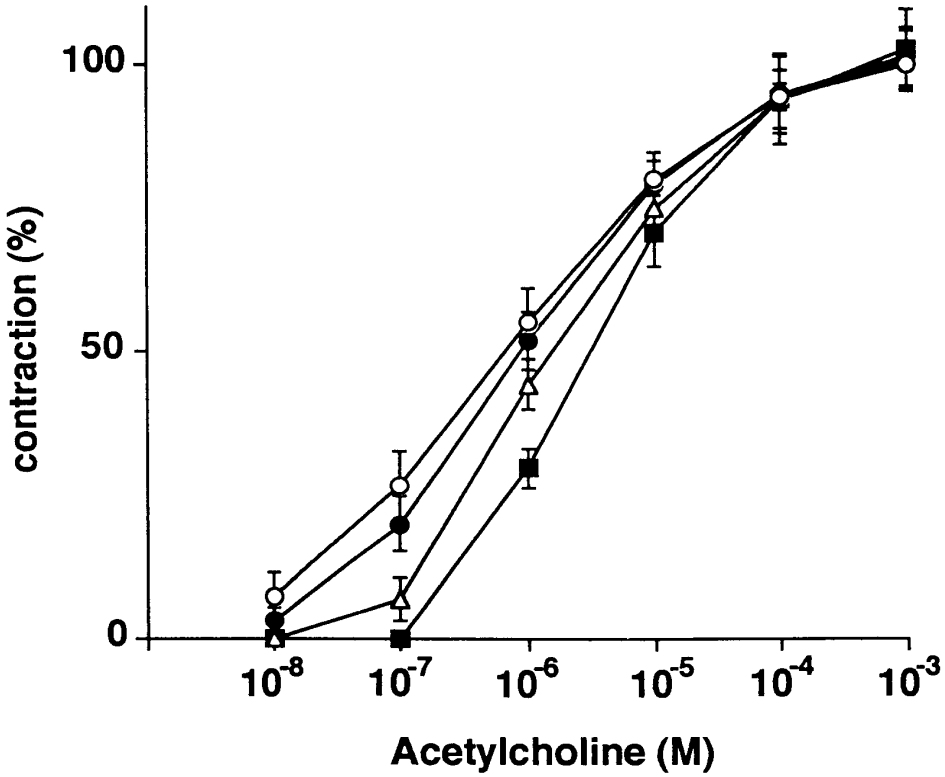

Fig. 5. The inhibitory effect of lead on cumulative dose-response curves for ACh. The contraction induced by ACh at each dose is expressed as a percentage of that evoked by 1 mM ACh in the absence of lead. Symbols indicate the mean values obtained from 4 to 6 experiments in the absence of lead (○) and presence of lead at concentrations of 5 μM (●), 10 μM (△) and 20 μM (■). After control responses were obtained, the preparation was exposed to lead for about 30 min and then ACh-induced contraction was observed in the presence of lead. ACh (log M) was added to the bathing solution cumulatively. Vertical bars represent SEM.

DISCUSSION

The present experiments showed that low concentrations of lead inhibited the contractions induced by vagal stimulation, transmural stimulation and ACh in smooth muscle segments isolated from chick proventriculus. Lead has been reported to inhibit transmitter release in the neuromuscular junction of the frog[5] and rat[8], in the cat superior cervical ganglia[4] and the rabbit saphenous artery[1] without any effects on the sensitivity of postsynaptic receptors to the transmitters. The contractile response to nerve stimulation has been demonstrated to be abolished by atropine, indicating the involvement of cholinergic transmission mechanism[7]. In the present experiments, although the responses to higher concentrations of ACh were not inhibited by lead even at higher concentrations, contractile responses to nerve stimulation and lower concentrations of ACh started to be inhibited by 3 μM and 6 μM lead, respectively. It is, therefore, suggested that the inhibitory effects of lead on neurally mediated contraction are related not only to the inhibition of transmitter release but also to the decreases in the responsiveness of proventricular smooth muscles to ACh.

Lead at 3 μM, the concentration of which inhibited the vagus nerve-smooth muscle transmission, corresponds to 0.6 ppm. It was reported that when mallard ducks were orally administered 4 lead shotgun pellets, the blood levels of lead attained over 4 ppm and the demyelinating lesion in the vagus nerves developed on 9 days after the administration[2]. The concentration of 4 ppm was high enough for lead to block the vagally-mediated contraction in the chick proventriculus. Thus, lead may block the vagus nerve-smooth muscle transmission before the development of demyelination of the vagal nerve fibres. It seems likely that proventricular impaction occurring in lead poisoning results from the pre- and post-synaptic inhibition of the vagus nerve-smooth muscle transmission. Furthermore, proventricular impaction may delay the recovery from lead poisoning of waterfowl by causing long term residue of lead shot.

REFERENCES

1) COOPER, G. P. & STEINBERG, D. : Effects of cadmium and lead on adrenergic neuromuscular transmission in the rabbit. *Am. J. Physiol.* **232**: C128–C131, 1977.
2) HUNTER, B. & WOBESER, G. : Encephalopathy and peripheral neuropathy in lead-poisoned mallard ducks. *Avian Dis.* **24**: 169–178, 1979.
3) KOBER, T. E. & COOPER, G. P. : Lead competitively inhibits calcium-dependent synaptic transmission in the bullfrog sympathetic ganglion. *Nature* **262**: 704–705, 1976.
4) KOSTIAL, K. & VOUK, V. B. : Lead ions and synaptic transmission in the superior cervical ganglion of the cat. *Br. J. Pharmacol.* **12**: 219–222, 1957.
5) MANALIS, R. S. & COOPER, G. P. : Presynaptic and postsynaptic effects of lead at the frog neuromuscular junction. *Nature* **243**: 354–356, 1973.

6) NAKAZATO, Y., SATO, H., & OHGA, A. : Evidence for a neurogenic 'rebound' contraction of the smooth muscle of the chicken proventriculus. *Experientia* **26** : 50–51, 1970.
7) SENO, N., NAKAZATO, Y. & OHGA, A. : Presynaptic inhibitory effects of catecholamines on cholinergic transmission in the smooth muscle of the chick stomach. *Eur. J. Pharmacol.* **51** : 229–237, 1978.
8) SILBERGELD, E. K., FALES, J. T. & GOLDBERG, A. M. : Evidence for a junctional effect of lead on neuromuscular function. *Nature* **247** : 49–50, 1974.
9) WOBESER, G. W. : *Diseases of Wild Waterfowl.* Plenum Press, New York, 1981.