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LEAD IONS AND SYNAPTIC TRANSMISSION IN THE SUPERIOR CERVICAL GANGLION OF THE CAT

BY

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The perfused superior cervical ganglion of the cat has been used to study the effect of lead ions on synaptic transmission and on the relase of acetylcholine from preganglionic nerve endings. Lead ions, in concentrations of 5 to 40 μM./l., caused block of ganglionic transmission, and reduced the output of acetylcholine. Calcium ions (10 mM./l.) relieved the block produced by lead ions and restored the acetylcholine output. The presence of lead ions does not seem to change the sensitivity of ganglion cells to injected acetylcholine.

The present experiments were performed to test whether lead ions influence ganglionic transmission; a preliminary report has already appeared (Kostial, Vouk and Purec, 1954). Our investigations were limited to the influence of lead ions on the contractions of the nictitating membrane and on the output of acetylcholine (ACh.) in response to stimulation of pre- and postganglionic nerve fibres in perfused superior cervical ganglia of cats. The effect of increasing the concentration of calcium ions in the presence of lead ions was also studied.

METHODS

The cats were anaesthetized with chloralose and the superior cervical ganglion prepared for perfusion by the conventional method as modified by Perry (1953). The pre- and post-ganglionic trunks were stimulated at frequencies of 2 and 10 shocks/sec., and the contractions of the nictitating membrane recorded with an isotonic lever writing on a smoked paper. When ACh was to be collected, eserine sulphate (1:100,000) was added to the perfusion fluid and the postganglionic trunk was tied. Lead nitrate was added to Locke solution without altering the concentration of other components. In some experiments extra calcium chloride was added to the perfusion fluid; in others ACh (100 μg./ml.) was injected into the arterial cannula. On each change-over from one solution to another the dead space was flushed. Care was taken to avoid accumulation of fluid around the ganglion. ACh was assayed on the blood pressure of eviscerated cats anaesthetized with chloralose. The presence of lead ions in the perfusate did not appear to interfere with the assay of ACh.

RESULTS

Nictitating Membrane Contractions.—In order to test the influence of lead ions on ganglionic transmission, the concentration of lead in the perfusing fluid was gradually increased. Fig. 1 shows contractions of the nictitating membrane recorded during stimulation of the preganglionic nerve for 45 sec. at a frequency of 2 shocks/sec. In this experiment the superior cervical ganglion was perfused with Locke solution containing 2.4, 4.8 or 12.1 μM. of lead ions/l. Each perfusion with lead was followed by a perfusion with Locke

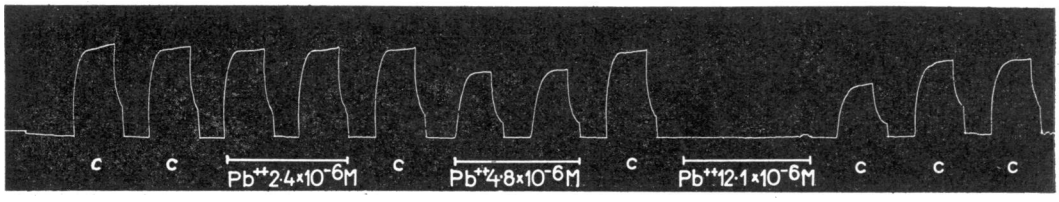

FIG. 1.—Contractions of the nictitating membrane of the cat in response to stimulation of the preganglionic trunk at 2/sec. for 45 sec. while perfusing the superior cervical ganglion with Locke solution (c) or with Locke solution containing increasing amounts of lead ions as shown.

solution. An effect of lead ions on the contractions of the nictitating membrane was first noticed at a concentration of 4.8 μM./l.; complete block occurred at 12.1 μM./l.

In other experiments, the concentration of lead ions causing failure of nictitating membrane contractions ranged from 9.6 to 40 μM./l. In many instances the recovery of the nictitating membrane response was only partial on subsequent perfusion with Locke solution.

The results of an experiment in which pre- and post-ganglionic fibres were stimulated alternately are presented in Fig. 2. The superior cervical ganglion was perfused with Locke solution containing lead ions in increasing concentrations. The usual effect of lead ions was observed on preganglionic nerve stimulation. There was, however, no effect of lead ions on the contractions of the nictitating membrane during postganglionic stimulation. Conduction in postganglionic fibres did not seem to be affected by addition of lead ions to the perfusion fluid. After 19.3 μM./l. of lead ions the response to low frequency stimulation of the preganglionic nerve was scarcely perceptible, although the response to higher frequency of stimulation was only slightly reduced. This comparatively slight impairment of ganglionic transmission at the higher frequency of stimulation was always observed. A similar phenomenon has been observed when ganglionic transmission is blocked by magnesium ion (Hutter and Kostial, 1954).

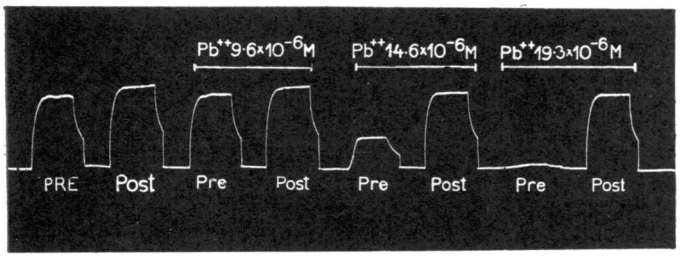

FIG. 2.—Contractions of the nictitating membrane in response to stimulation of pre- or post-ganglionic fibres at 2/sec. for 45 sec. Lead ions suppressed the response of the nictitating membrane to preganglionic stimulation, leaving the response to postganglionic stimulation unaffected.

Control experiments with sodium nitrate showed that nitrates in the concentrations used here had no influence on nictitating membrane contractions (Kostial *et al.*, 1954).

Effect of Lead Ions on Acetylcholine Output.—The influence of lead ions on the output of ACh is shown in Fig. 3 in which each column represents the amount of ACh collected over a period of 5 min. while stimulating the preganglionic nerve at a frequency of 2/sec. Fig. 3*a* gives the results of an experiment with 2.4 μM. of lead ions. The first sample was taken after perfusing the ganglion with normal Locke solution for 12 min.; 2.4 μM./l. Pb^{++} was then added to the perfusion fluid and, after an exposure of 12 min., a 5 min. sample was taken. The third sample was collected 12 min. after changing over to Locke solution. A slight decrease in the output of ACh was noticeable. Fig. 3*b* shows an experiment with 4.8 μM./l. of Pb^{++} which caused the ACh output to fall to about 35% of its initial value, but recovery was only partial. With higher concentrations of lead ions the perfusate contained only very little ACh (Fig. 3*c* and *d*).

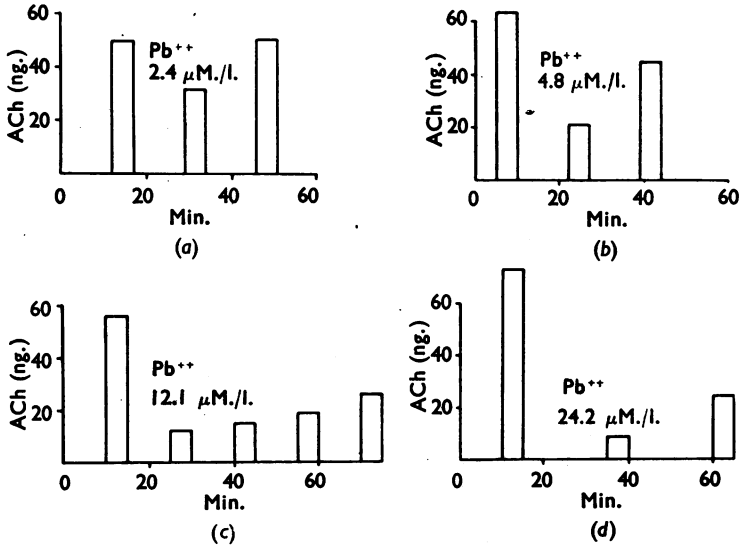

FIG. 3.—Ganglion perfused with Locke solution (eserine 10^{-5}). Each column represents the amount of ACh released during 5 min. of stimulation of the preganglionic nerve at 2/sec. In each section the ACh outputs before and after exposure to lead ions are shown. The ganglion was perfused with Locke solution containing lead ions in (*a*) 2.4 μM./l. for 17 min.; in (*b*) 4.8 μM./l. for 17 min.; in (*c*) 12.1 μM./l. for 30 min. (the second sample being taken 10 min. and the third 25 min. after exposure to lead), and (*d*) 24.2 μM./l. for 25 min.

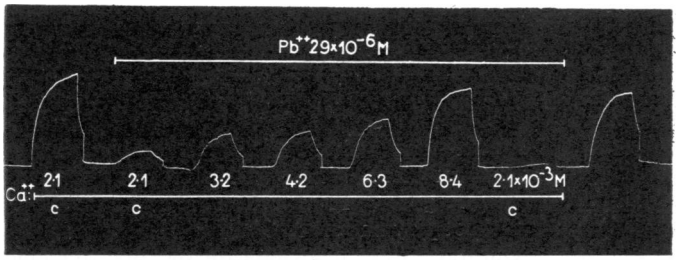

FIG. 4.—Contractions of the nictitating membrane in response to stimulation of preganglionic nerve. By increasing the concentration of calcium ions the block caused by the presence of lead ions was gradually relieved. C=Perfusion with Locke solution containing the normal concentration of calcium ions (2.1×10^{-3}M). The calcium ion concentration was raised to 3.2, 4.2, 6.3, and 8.4×10^{-3}M, and then returned to normal while perfusing the ganglion with Locke solution containing 29×10^{-6}M, lead ion concentration.

Antagonistic Effect of Calcium Ions.—The blocking action of lead ions is almost completely relieved by addition of calcium ions. When gradually increasing the concentration of calcium ions in the perfusion fluid containing 29 μM./l. of lead ions, complete recovery of nictitating membrane contractions to preganglionic stimulation was observed at a concentration of 8.4 mM. of calcium ions/l. (Fig. 4).

The antagonistic effect of calcium ions was also observed in experiments in which ACh output from the superior cervical ganglion was measured. Addition of 24.2 μM./l. of lead ions caused a pronounced reduction of the ACh output, with practically no recovery on returning to perfusion with Locke solution (Fig. 5, col. 3). By contrast, addition of five times the normal amount of calcium (10.5 mM.) to the perfusing solution containing 24.2 μM./l. of Pb^{++} restored the output of ACh to nearly its initial value. A similar restorative effect of calcium has been observed when block is produced by magnesium ions (Hutter and Kostial, 1954).

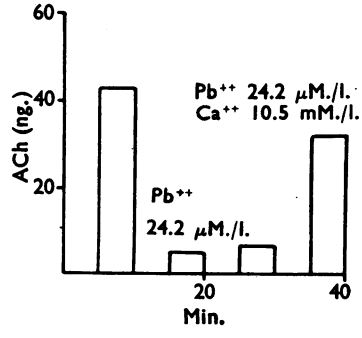

FIG. 5.—Ganglion perfused with Locke solution (eserine $\times 10^{-5}$). Each column represents the amount of ACh released during 5 min. of stimulation of the preganglionic nerve at 2/sec. 24.2 μM./l. Pb^{++} was present during collection of sample 2. 24.2 μM./l. Pb^{++} and 10.5 mM./l. Ca^{++} was present during collection of sample 4.

Effect of Acetylcholine on Nictitating Membrane Contractions in Presence of Lead Ions.—Injection of ACh (10 μg. in 0.1 ml.) into the arterial cannula induced contractions of the nictitating membrane in a ganglion blocked to preganglionic stimulation by lead ions (Fig. 6). The sensitivity of ganglion cells to applied ACh did not appear to be altered by the presence of lead ions in the perfusion fluid.

DISCUSSION

Addition of lead ions to the perfusion fluid caused a partial or complete block of synaptic transmission indicated by failure of nictitating membrane contractions on preganglionic nerve stimulation. This failure in transmission was accompanied by a reduced output of ACh from the superior cervical ganglion. The effects of lead ions could be reversed by increasing the calcium content of the perfusion fluid.

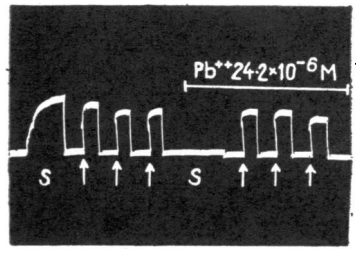

FIG. 6.—Responses of nictitating membrane to intra-arterial injections of ACh (10 μg. in 0.1 ml.) to the superior cervical ganglion at arrows. Three injections were made while perfusing with normal Locke solution, three after addition of 24.2×10^{-6}M. Pb^{++} to the Locke solution. S=Contractions in response to preganglionic nerve stimulation.

Several heavy metal ions (Cd^{++}, Cu^{++}, Ag^{+}, Hg^{++}, UO_2^{++}) cause an impairment of nerve conduction (Del Castillo and Hufschmidt, 1951). In our experiments, lead ions in concentrations causing a block of synaptic transmission did not seem to have an effect on nerve conduction. Contractions of the nictitating membrane occurred on stimulation of the postganglionic trunk in spite of the presence of lead ions. Furthermore, addition of ACh to the perfusion fluid containing enough lead to cause failure in synaptic transmission evoked contractions of the membrane.

The effects of lead ions on synaptic transmission were noticeable after 1 to 3 min. Mortensen and

Kellog (1944) found lead inside cells a few minutes after an intravenous injection. The temperature dependence of this reaction made these authors interpret the entrance of lead into the cell as an active chemical process. However, the speed of action of heavy metals points more to a cell surface action than to a diffusion process across the cell membrane. According to Davson (1951) heavy metals seem capable of blocking active groups in the cell membrane in accordance with their ability to inhibit enzyme catalysed reactions.

The antagonizing action of calcium ions might be explained by assuming a competitive action of lead and calcium ions for the same protein molecules, lead forming stronger protein complexes than calcium. According to Fatt (1954) calcium ions are essential for the functioning of an enzyme-like factor in the cell membrane, responsible for liberation of ACh from nerve endings. An excess of calcium ions might eliminate lead ions from their protein complexes and restore the normal activity of the enzyme-like factor in the membrane. It is very difficult to form any conclusive opinion on that subject, since very little is known about lead complexes with proteins (Cohn, Surgenor, Schmidt, Batchelor, Isliker and Alameri, 1953).

Lead ions inhibit resynthesis of phosphocreatine in the muscle (Steiman, 1939). Since we know that resynthesis of phosphocreatine also plays an essential role in the metabolism of ACh, we might assume that lead affects ACh synthesis. Del Castillo and Hufschmidt (1951) tried to explain the effect of heavy metals on nervous activity by their inhibiting action on SH-groups in the cell membrane. The SH-group is found to be a functional group of the co-enzyme A which is essential for acetylation of choline (Reisberg, 1954). Our results, however, cannot be explained by an effect of lead ions on ACh synthesis alone, since an impairment of transmission occurred before the store of ACh could be depleted (Perry, 1953).

Lead ions caused the same effect with and without addition of eserine to the perfusion fluid. The action of lead on cholinesterase (Frommel, Herschberg and Piquet, 1943, 1944), therefore, could not play an essential role in our experiments.

Our results seem to indicate the preganglionic nerve endings as the main site of action of lead ions. The influence of lead on nerve conduction, on ACh synthesis and on cholinesterase activity cannot be, however, entirely eliminated, but they do not seem to have an essential influence on our results.

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