The Action of Mercury Ions on the Release of Acetylcholine from Presynaptic Nerve Endings

Lead and manganese ions block synaptic transmission by reducing the release of acetylcholine from presynaptic nerve endings[1-3]. There are good reasons to believe that this action is the result of interference by the foreign ions in the extracellular fluid with the entry of extra-cellular calcium ions into the presynaptic nerve endings[4]. In experiments on neuromuscular junction it has recently been shown that lead, manganese and other metal ions also bring about a spontaneous release of acetylcholine[5-8]. This second action is probably of intracellular location and it may consist of either the substitution of the foreign metal ion for calcium at the site of transmitter release, or the displacement of calcium from intracellular stores[9,8]. In view of these recent results the question arises whether foreign metal ions also have a dual action on the presynaptic nerve endings in the sympathetic ganglion. Under the experimental conditions which have so far been employed the possible intracellular action of lead and manganese was not powerful enough to be detectable in ganglion perfusion experiments. In experiments with mercury, however, we have observed a great increase in the spontaneous release of transmitter which deserves description in view of the fact that the action of this ion has not so far been studied at other junctions.

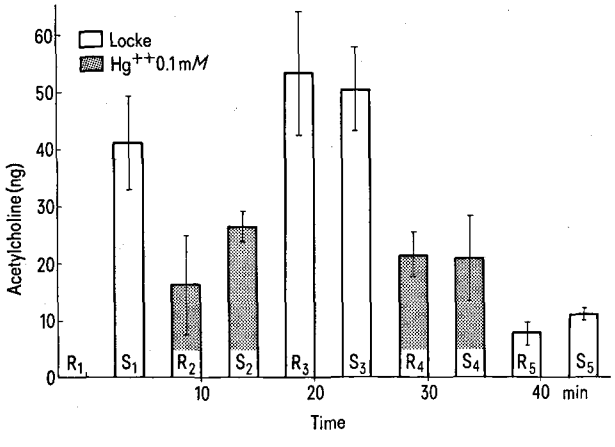

Fig. 1. Ganglion perfused with Locke solution (eserine 10^{-5}) to which mercury chloride was added (0.1 m*M*). Each column represents the acetylcholine released during 5 min of stimulation of the preganglionic nerve at 2 Hz (S_1 to S_5) and during the resting period (R_1 to R_5). The values represent the mean of 2 experiments.

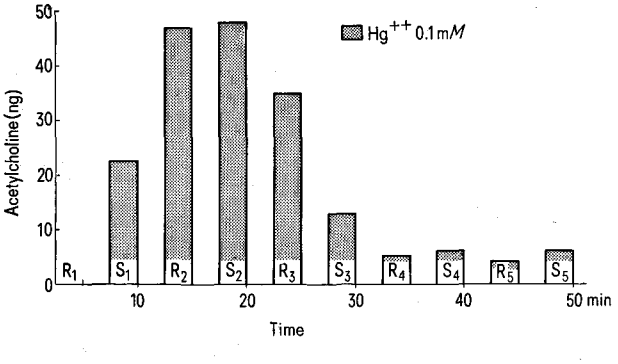

Fig. 2. Ganglion perfused with Locke solution (eserine 10^{-5}). Mercury chloride (0.1 m*M*) was added to Locke solution in samples R_2, S_2, R_4 and S_4. Each column represents the acetylcholine released during 5 min of stimulation of the preganglionic nerve at 2 Hz (S_1 to S_5) and during the 5 min resting period (R_1 to R_5). Values represent the arithmetic mean of 3 experiments.

Methods and results. The superior cervical ganglion of the cat was perfused as described for previous experiments[3]. In the experiment in Figure 1 the normal relation between the periods of stimulation and transmitter release[10] breaks down after administration of mercuric chloride. Thus some acetylcholine appears already in the first sample after admission of the mercuric chloride solution, and upon return to Locke solution the first sample collected in the absence of stimulation contained as much acetylcholine as the second sample collected during stimulation. In the later stages of the experiment the output of acetylcholine declined again regardless of stimulation.

In two other experiments (Figure 2) the fresh preparation was perfused with mercuric chloride. In these experiments no acetylcholine was detectable in the first sample collected from the resting preparation, but a large spontaneous release was found to occur during the rest period subsequent to the first period of stimulation. Subsequent periods of stimulation caused no further enhancement in acetylcholine release which then followed essentially the same pattern as in Figure 1. When unstimulated ganglia were perfused with mercury solution the spontaneous release appeared gradually after a variable interval (Table).

Discussion. KOSTIAL and VOUK[11] and VOUK, KOSTIAL and HEFER-ŠLAT[12] showed that mercury ions block synaptic transmission. They concluded that the block of transmission was principally due to an irreversible postsynaptic action of mercury ions. However, they

The effect of mercury on the spontaneous release of acetylcholine in the superior cervical ganglion of the cat

	Experiment 1	Experiment 2	Experiment 3
Sample 1	< 5	< 5	< 5
Sample 2	< 5	13	36
Sample 3	9	40	78

Each figure represents the acetylcholine output (ng) during 5 min while perfusing the ganglion with Locke solution containing mercury (0.1 m*M*). The preganglionic trunk was not stimulated. 3 consecutive 5 min samples were collected.

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presumed mercury ions to have also a reversible presynaptic action. In the light of the experiments illustrated in Figure 1, it must now be concluded that the transmitter release previously observed on readmission of Locke solution [12] represented in fact the long-lasting spontaneous release induced by the treatment with mercury and not the restoration of the response to preganglionic stimulation as was initially presumed.

On the basis of the information presented here the possibility cannot be discounted that both pre- and post-ganglionic effects of mercury ions are due to a generalized depolarizing action. However, in experiments in which the postganglionic nerve was left untied we have never observed a transient contraction of the nictitating membrane such as might be expected if the cell bodies of perfused ganglia underwent depolarization. Furthermore it proved possible to elicit contractions of the nictitating membrane by postganglionic stimulation in the presence of 0.1 m*M* mercury ions. CASTILLO and HUFSCHMIDT [13] found 250 μ*M* mercury to be the minimum concentration causing inexcitability of motor nerve fibres. The rapid reversibility of this effect by the application of thiols points to an increase in the threshold voltage rather than to a generalized depolarization as the cause of inexcitability. Nevertheless it is clearly desirable, in view of the toxicological importance of mercury, that their mode of action on synaptic transmission be investigated further by electrophysiological means [14].

Zusammenfassung. In den oberen zervikalen Ganglien der Katze verursacht eine Perfusion mit Locke-Lösung mit 0.1 m*M* Quecksilber Chlorid eine spontane Freisetzung von Acetylcholin. Dieses Phänomen wird vom Standpunkt der bekannten Wirkung anderer Metalionen bei der Freisetzung von Transmittern diskutiert.

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[14] We are very grateful to Professor O. F. HUTTER for helpful discussion of our work. This work was supported by a research grant from the U.S. Environmental Protection Agency.

Reserpine-Induced Changes in the Uptake and Distribution of Radiolabelled Calcium and Magnesium in the Brain and Pituitary Gland of the Rat

Little is known about the effects of various drugs affecting transmitter release on the in vivo movements of calcium and magnesium in brain tissue. Reserpine is well known to exert an influence on the uptake and release of transmitters and induces a long-lasting reduction of serotonin, norepinephrine and dopamine in the brain and peripheral stores [1, 2]. Amine depletion is thought to be due to changes in the permeability of neuronal membranes [3]. In the present report we present the effects of reserpine on the in vivo uptake of radiolabelled calcium and magnesium by certain areas of the rat brain and pituitary gland. The procedure of earlier investigations with some modifications was followed [4, 5]. Nonfasting white male rats (Sprague-Dawley) weighing 250–300 g and housed in individual cages were used in the study. The animals were injected for 3 days either with saline (0.2 ml, i.m.) for the control group, or with reserpine (2 mg/kg, i.m.) for the test group. The injection schedule on the last experimental day was the following: each animal received saline or reserpine respectively, 4 h prior to injection of the radiolabelled material. Each animal was anaesthetized with pentobarbital and received into the carotid artery a dose of 1 μCi of ^{45}Ca and ^{28}Mg (in 0.2 ml of Ringer's solution buffered to a pH of 7.56 with 4 m*M* HEPES buffer). Due to the short half-life of ^{28}Mg the injection solution was calibrated to be 1 μCi/0.2 ml of ^{45}Ca and ^{28}Mg at the beginning of the experiment. The injection was followed by decapitation in 15 sec. Following decapitation, the brain was quickly dissected free and the following tissues placed into tared scintillation vials and weighed: cortex, hippocampus, cerebellum, thalamus, superior colliculus, medulla and the pituitary gland. 1 ml aliquots of tissue solubilizer (Soluene-350, Packard) were added to each tissue vial and tissues digested within 2 h, after which 10 ml aliquots of a scintillation mixture (Dimilume, Packard) were added to each vial. The β-radiation of ^{28}Mg and ^{45}Ca was measured with a Beckman LS-200

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Table I. Uptake of ^{45}Ca by different brain areas and pituitary gland in rats following intracarotid injection of 1 μCi of ^{45}Ca

Tissue	*N*	Control	Reserpine	Statistical significance (*p*)
Cortex	82	3,311 ± 173	4,806 ± 507	< 0.004
Hippocampus	80	4,091 ± 342	7,907 ± 948	< 0.000
Thalamus	80	3,753 ± 511	6,086 ± 1,036	< 0.038
Superior colliculus	41	6,811 ± 990	9,127 ± 1,228	NS
Cerebellum	79	6,364 ± 528	7,644 ± 554	NS
Medulla	41	10,283 ± 1,848	8,319 ± 1,066	NS
Pituitary gland	40	179,508 ± 39,934	542,381 ± 159,304	< 0.018

The radioactivity is expressed as dpm/g of wet tissues. Values are expressed as means ± S.E.M.