EXPERIMENTAL STUDIES ON DETOXICATION OF MERCURY SALTS BY MEANS OF ETHYLENEDIAMINETETRA-ACETIC ACID

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Ethylenediaminetetraacetic acid (EDTA) reacts with the ions of many heavy metals, forming stable water-soluble complexes, and in this way it inactivates the metals, and facilitates their elimination from the organism. This property has led to its application to the treatment of heavy metal poisoning: lead [6, 13, 14], vanadium [12] (Mitchell, 1953), plutonium [10], manganese (Radier, 1954), and others, and for the inactivation of cobalt [3, 8] (Child, 1951, Belgova, 1956) and cadmium [2], and for acceleration of elimination of radioactive elements from the body, such as yttrium [9, 15].

The object of the present research was to investigate the possibility of inactivating mercury ions by means of EDTA, and to find the necessary quantitative ratios between EDTA and mercury; this problem has not been elucidated in the literature.

For this purpose we selected experimental conditions such that biologically detectable amounts of mercury ion were as small as possible, in order to facilitate the determination of the ratio of EDTA to mercury needed to suppress the toxic action of the latter. A suitable experimental procedure was to prevent development of carbocholine effects on frog heart by means of one of the salts of mercury (mercuric chloride) [1].

The reactivity of an isolated frog's heart to the same concentration of carbocholine (1 : 4,000,000 – 100,000,000) was tested 3 times in each experiment (the carbocholine test).

First carbocholine test. After recording contractions of the heart for 5-10 minutes carbocholine was introduced into the cannula, causing either a reduction of the amplitude of the contractions, or their total arrest. The heart was then washed out with Ringer solution for a minute, to remove carbocholine. Second carbocholine test: after the amplitude of the contractions had returned to the initial level calcium EDTA was introduced into the cannula (concentration, 1 : 10,000 – 20,000), followed after 5-10 minutes by a mercuric chloride solution (1 : 1,100,000); in some experiments mixtures of mercuric chloride and EDTA solutions, of the above concentrations, were made 30-60 minutes earlier, and were added to the perfusion fluid. Reactivity of the heart to carbocholine was again tested (for 1 minute), 10-15 minutes after perfusion with mercuric chloride or mercuric chloride-EDTA mixture. The extent of inactivation of the mercury ion by EDTA was assessed from the intensity of the carbocholine reaction; if it approached that of the first test it followed that the mercuric chloride effect was abolished, indicating binding of mercury ions, while if the carbocholine reaction was weakened or abolished it followed that mercury ions were present.

Third carbocholine test: this was performed after washing out EDTA, mercuric chloride, and carbocholine. Mercuric chloride was perfused, at the same concentration as in the preceding experiment, and was followed after 10-15 minutes by carbocholine. The carbocholine reaction was either absent, or was very weak, in this test. The mercuric chloride reaction was irreversible, and for this reason this test had to be the last of each series. The third test was not performed in many of the experiments, when the second carbocholine reaction was found to be suppressed.

In all, we performed 20 experiments, involving 62 tests.

The molar proportions of EDTA to mercuric chloride were 6, 12, 24, 48, and 120.

EXPERIMENTAL RESULTS

Statistical treatment of the experimental data showed that the effect of mercuric chloride is abolished when the molar ratio of EDTA to mercuric chloride is 120:1. Inactivation of mercuric chloride is not observed when this ratio is lowered to 48:1.

The results found for the isolated heart preparation led us to investigate the possibility of applying EDTA to inactivation of mercury ions in the intact organism. These experiments were performed on 50 adolescent female albino mice, weighing 18-22 g. Mercuric chloride was given by intraperitoneal injection, at a dosage level of 10 μg per g body weight, and calcium EDTA by intravenous injection (dose, 400 μg/g) or subcutaneously (dose, 1 mg/g). The respective molar ratios of EDTA to mercuric chloride were, accordingly, 60:1 and 100:1. At these dosage levels, calcium EDTA alone does not produce any toxic symptoms in mice (10 mice). Injection of mercuric chloride alone, to a control group, caused the death of the animals within 2-3 days, with symptoms of acute apnea (10 mice). Previous administration of EDTA (20-30 minutes before mercuric chloride) did not prevent the death of the animals (30 mice).

DISCUSSION OF RESULTS

The toxic effect of mercuric chloride on an isolated frog's heart was abolished if it had previously been mixed with EDTA, or if the heart had previously been perfused with EDTA. This could only be achieved, however; by the use of a considerable excess of EDTA (120 M EDTA : M $HgCl_2$). Inactivation of the mercury ion could not be achieved in the whole organism by injection of EDTA in approximately the same molar proportions.

Our results indicate that EDTA binds mercury ions much less firmly than it does other metals. Thus we have shown in earlier papers that cadmium [2] and cobalt[3] are inactivated by EDTA at molar ratios of 1 and 2, respectively. It is known, on the other hand, that the toxic effects of mercuric chloride can be abolished by compounds containing sulfhydryl groups, such as mercaptosuccinate [1, 5, 11] and cysteine [4]. It may hence be concluded that mercury ion is much more firmly bound by sulfhydryl groups, for which reason compounds containing such groups are better antidotes to mercury poisoning than is EDTA. This view is supported by the findings of Bell (1955), who studied the effects of EDTA and dimercaprol (BAL) on elimination of mercury and lead in a case of chronic poisoning. EDTA caused a slight fall in excretion of mercury, whereas dimercaprol greatly increased it.

SUMMARY

It was experimentally proved that mercury may be fixated with the aid of ethylendiaminetetraacetic acid. The latter, likewise, promotes excretion of mercury from the body. It was established in experiments on isolated frog's heart that the ratio of the quantity of sublimate and ethylendiaminetetraacetic acid should be 1:120 for total fixation of mercury. However, it was impossible to fixate mercury in the intact organism, even in presence of this ratio.

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