THE EFFECT OF SALTS OF COPPER AND MANGANESE ON THE CHOLINERGIC SYSTEMS OF THE HEART

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(Received September 9, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

Copper and manganese are known to possess parasympatheticotropic activity. Copper chloride, for instance, depresses the work of the heart, and moreover, the degree of this action depends on the concentration of copper; copper salts in low concentrations increase, and in high concentrations decrease the inhibitory action of the vagus nerve on the frog's and rabbit's heart [7]. Potassium permanganate is another substance with a depressing action on the work of the isolated heart [6]. Many authors have shown that manganese and copper salts intensify the hypoglycemic action of insulin, diminish adrenalin and alimentary hyperglycemia, lower the blood sugar level in normal and diabetic subjects, and so on [1, 2, 8 and others]. The authors cited do not, however, explain the mechanism of the biological action of copper and manganese salts.

In the present work we attempted to discover the effect of salts of copper and manganese on the regulation of the cardiac activity, by studying the level of excitation of the cholinergic systems of the heart.

EXPERIMENTAL METHOD

The experiments were carried out on the frog's heart, isolated by Straub's method, during the autumn-winter period. Solutions of copper and manganese chlorides were made up in Ringer's solution before the beginning of the experiment. In this research we used copper salts in concentrations (calculated as the pure metal) of 1×10^{-8} to 1×10^{-5} and manganese salts in concentrations of 5×10^{-8} to 1×10^{-4}.

EXPERIMENTAL RESULTS

In the first series of experiments we studied the effect of these salts of copper and manganese on the frequency and amplitude of the cardiac contractions. It was found that the copper solutions in dilutions of 1×10^{-8}, when acting for a considerable time, caused no appreciable changes in the mechanogram of the heart. With a concentration of copper of 1×10^{-7} a gradual and very slight decrease was observed in the amplitude of the cardiac contractions, which reached only 20-30% of the initial value after 5 minutes. The amplitude of the cardiac contractions was sharply decreased as a result of administration of copper in a concentration of 1×10^{-6}. Under these conditions both a weakening of the systole and a decrease in the diastolic relaxation were observed, although the latter was rather less apparent. The height of the cardiac contractions fell by 50% in the course of a minute. The heart rate remained as before even when considerably weakened. Rinsing the heart with Ringer's solution restored the activity of the heart to its initial level with varying speed.

Investigation of the changes in cardiac activity under the influence of manganese salts showed that the prolonged action of manganese in a dilution of 5×10^{-8} caused a very slight fall in the amplitude of the cardiac contractions of not more than 20% in the course of 5 minutes. Under the influence of a 5×10^{-7} solution of

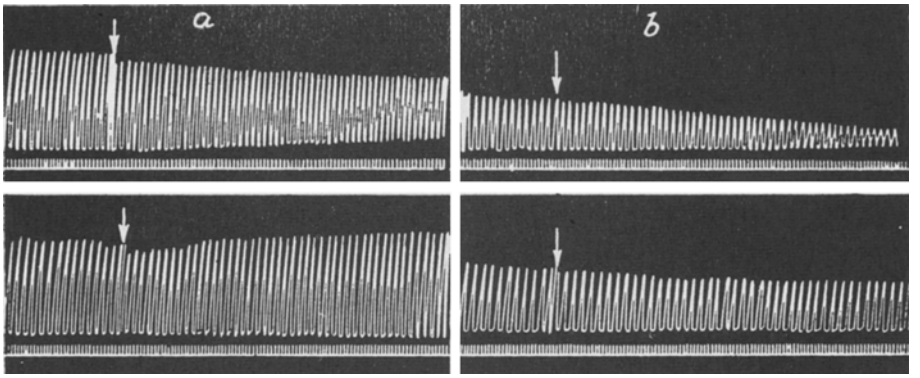

Fig. 1. The action of copper and manganese salts on the isolated frog's heart. a) action of copper (1×10^{-7}) after perfusion with Ringer's solution (I) and after atropinization of the heart (II); b) action of manganese (5×10^{-7}) after perfusion with Ringer's solution (I) and after atropinization (II) (the arrows indicate the beginning of perfusion with the test solution).

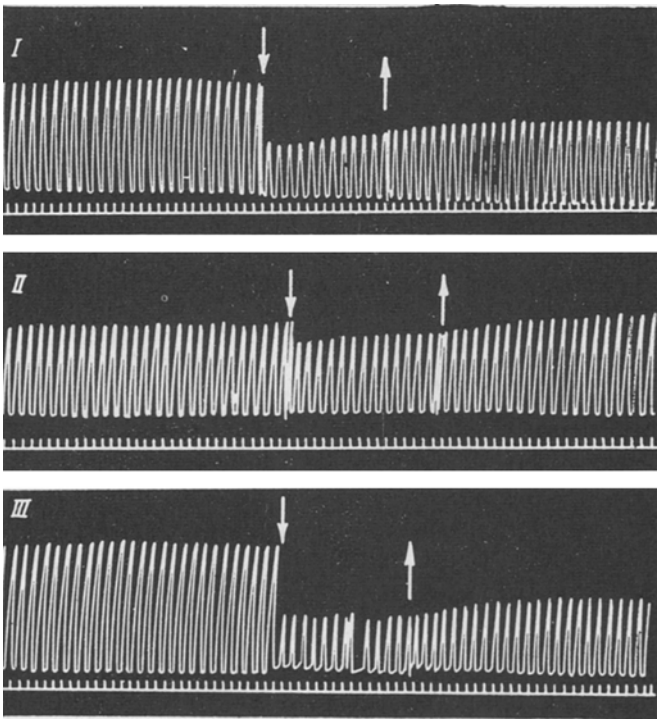

Fig. 2. Changes in the inhibitory action of acetylcholine on the heart under the influence of copper and manganese salts. I) action of 1×10^{-8} acetylcholine on the heart in control experiments; II) action of the same concentration of acetylcholine after perfusion of the heart with 1×10^{-7} copper solution for 5 minutes; III) the same after perfusion of the heart with 5×10^{-8} manganese solution for 5 minutes.

manganese the weakening of the cardiac contractions also developed gradually, but it was more pronounced and reached on the average 50% (Fig. 1, b, I). In a concentration of 1×10^{-6}, manganese caused a rapid fall in the amplitude of the cardiac contractions of 60-70% of their initial value, on account of a diminution of both systole and diastole. The heart rate was appreciably unchanged in all the experiments.

The electrocardiogram of the isolated heart under the influence of copper and manganese salts in the above concentrations showed no specific changes in its automatism and conductivity.

In the second series of experiments we studied the effect of copper and manganese salts on the heart aganist a background of the action of atropine. A 1: 10 000 solution of atropine was applied for 5 minutes, after which the effect of copper or manganese was tested. It was found that copper, in a dilution of 1×10^{-7}, did not cause any decrease in the amplitude of the contractions of the atropinized heart (Fig. 1, a II). In a concentration of 1×10^{-6}, copper led to an insignificant depression of the action of the heart in a minority of the experiments and only in a dilution of 1×10^{-5} did it constantly evoke a decrease in the amplitude of the contractions by 25-30%. In order to obtain an effect on the atropinized heart it was thus necessary to increase the concentration of copper almost a hundredfold.

Similar results were obtained by the action of manganese on the atropinized heart. During the action of manganese in a concentration of 5×10^{-8} to 1×10^{-5} the activity of the atropinized heart was completely unaltered (Fig. 1, b, II). Only in a dilution of 1×10^{-4} did manganese cause a gradual decrease of 50% in the amplitude of the cardiac contractions. It is clear from these results that the concentrations of manganese which depress the activity of the atropinized heart were increased roughly 200 times. From 10 to 30 minutes after rinsing the atropine from the heart, the previous sensitivity of the heart to copper and manganese was gradually restored.

In the third series of experiments we investigated the changes in the reaction of the heart to acetylcholine under the influence of copper and manganese salts. The solution of acetylcholine 1×10^{-8}, which we injected into the cannula, acted on the heart for 20 seconds. In most experiments in which copper solutions acted on the heart some degree of weakening of the reaction of the heart to acetylcholine was observed, depending on the concentration and the duration of action of the copper. Whereas in the control experiments the introduction of a 1×10^{-8} solution of acetylcholine caused, in 2-5 seconds, a decrease of 50-70% of the initial value in the amplitude of the cardiac contractions, after the prolonged action of high dilutions of copper salts (1×10^{-7}) or after the action of higher concentrations for a short period of time (up to 5 seconds), the same solution of acetylcholine caused a decrease of 20-40% in the amplitude of the cardiac contractions. The more prolonged action (10-15 minutes) of high concentrations of copper (1×10^{-5}), accompanied by marked changes in the mechanogram of the heart, completely abolished the reaction of the heart to acetylcholine (Fig. 2). In two experiments a small increase was observed in the cardiac contractions in response to the injection of acetylcholine. During the action of small concentrations of copper (1×10^{-7}), in some experiments a considerable increase was observed in the effect from the injection of acetylcholine.

Rinsing the heart with Ringer's solution after the action of small concentrations of copper caused the partial restoration of its reaction to acetylcholine, whereas after giving high concentrations of copper the absence of a reaction to acetylcholine was irreversible.

The foregoing refers to the effect of acetylcholine in dilutions of 1×10^{-8} to 1×10^{-9}. Increasing the concentration of acetylcholine a hundredfold causes arrest of the heart irrespective of the preliminary action of copper.

In order to test the character of the interaction between copper salts and acetylcholine, we carried out control experiments. After testing the reaction of the heart to acetylcholine and washing this out, we replaced the Ringer's solution in the cannula with a mixture of copper and acetylcholine solutions prepared 5-10 minutes before administration (in the same concentrations as in the previous test). It was found this mixture caused the same depression of the activity of the heart as did acetylcholine alone at the beginning of the experiment.

The study of the effect of manganese salts on the sensitivity of the heart to acetylcholine showed changes of an opposite character. Manganese in a dilution of 5×10^{-8}, which by itself did not appreciably alter the amplitude of the cardiac contractions, caused an obvious increase in the inhibitory action of acetylcholine. The decrease in the amplitude of the cardiac contractions in response to injection of acetylcholine after the action of manganese developed more quickly and was from 2 to 4 times more pronounced than that in response to injection of acetylcholine alone. The activity of the heart was also restored more slowly after the acetylcholine had been rinsed out (Fig. 2, III). With an increase in the concentration of manganese, its strengthening action on the inhibitory effect of acetylcholine was enhanced.

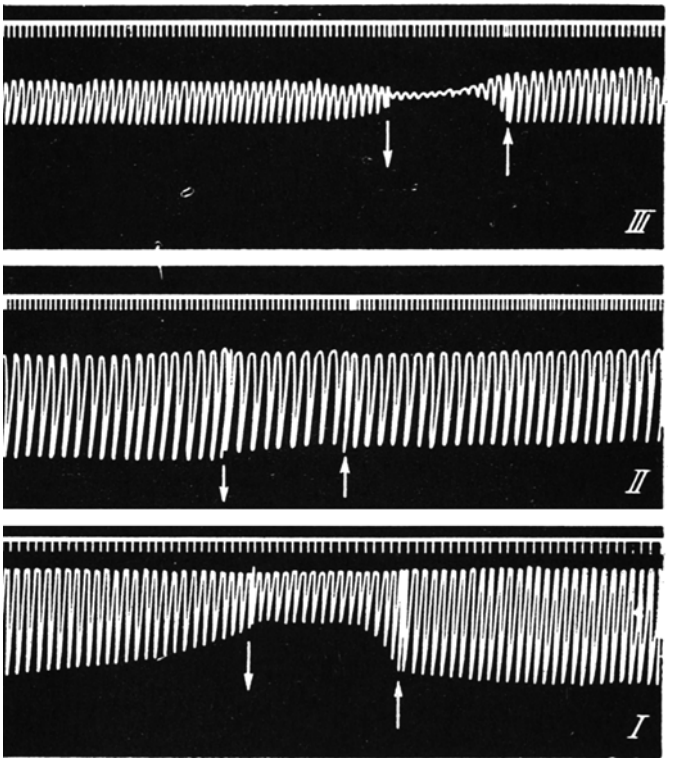

Fig. 3. The effect of cysteine on the cholinolytic action of copper. 1) action of 1×10^{-8} acetylcholine on the heart; II) action of the same concentration of acetylcholine after preliminary perfusion of the heart with a 1×10^{-7} copper solution; III) the same after preliminary perfusion of the heart with a 1×10^{-7} copper solution and 1% cysteine.

In the fourth series of experiments we studied the effect of compounds which acted as donors of sulfhydryl groups on the action of copper salts. As compounds of this type we used cysteine and urea which, from findings in Kh. S. Koshtoyants's [4] laboratory, restore the sensitivity of the heart to neurohumoral influences when it has been altered by blocking of the sulfhydryl groups by the thiol poisons. For this purpose we first acted on the heart with copper solutions, leading to a decrease in the reaction of the heart to acetylcholine or to its disappearance. Against this background, the copper solution in the cannula was replaced by 1% solution of cysteine, and the action of acetylcholine was again tested after 30-60 seconds. It was found that, after the action of cysteine solution, acetylcholine caused inhibition of the action of the heart, just as in the control experiments (Fig. 3). At the same time it has to be pointed out that after the action of urea (1-5% solutions) we were unable to observe any recovery of the reactivity of the heart to acetylcholine after its depression of copper salts.

Our investigations showed that copper and manganese salts, in low concentrations, not only affect the activity of the heart but also cause marked changes in the reactivity of the heart to acetylcholine. Both elements show a similar negative, inotropic action on the heart, characterized both by depression of systole and by incomplete relaxation during diastole. The fact that this effect was abolished by preliminary atropinization of the heart indicates its vagotropic character, i.e., this is in agreement with the reports in the literature of parasympathicotropic action of copper and manganese. Investigation of the changes in the reactivity of the heart to acetylcholine, however, revealed a difference in the mechanism of action of copper and manganese. It is clear from the results described that manganese, while it exerts a vagotropic effect on the heart, at the same time increases the level of excitation of its cholinergic systems. Copper, however, which also exerts a vagotropic effect, leads to a sharp depression of the level of excitation of the cholinergic systems. The experiments with cysteine showed that the depression of the level of excitation of the cholinergic system is connected with the blocking of the sulfhydryl groups by copper. Our results are in agreement with experimental findings that copper accelerates the oxidation of sulfhydryl into disulfide groups [3].

SUMMARY

Copper and manganese salts, in small concentrations (5×10^{-8} to 1×10^{-6}), thus causes a decrease in the amplitude of the contractions of the isolated frog's heart; under these circumstances the systolic contractions is weakened and the diastolic relaxation is diminished. The heart rate is unaffected. This action of copper and manganese salts does not take place after preliminary atropinization of the heart. The action of copper is to cause a decrease in the reactivity of the heart to acetylcholine, connected with blocking of the sulfhydryl groups by the copper; manganese, on the contrary, increases the sensitivity of the heart to acetylcholine.

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