PHARMACOLOGY

BIOCHEMICAL METHODS OF REMOVAL OF THE BLOCKING ACTION OF METHYLENE BLUE ON THE VAGUS NERVE

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The transmission of excitation along nerves is known to be disturbed by chemical agents acting on any of the links in the chain of metabolism. One of the substances which blocks the transmission of nervous excitation (or inhibition) from the vagus nerve to the heart is methylene blue.

Koskowski and Maigre [9] observed that methylene blue causes a paralytic condition of the vagus nerve, and M. L. Belen'kii demonstrated that methylene blue inhibits the action of acetylcholine on the frog's heart. I. A. Keder-Stepanova [2] showed that methylene blue in a concentration of $2 \cdot 10^{-5}$ g/ml causes reversible inhibition of the action of the vagus nerve on the frog's heart but large concentrations of this substance have an irreversible effect. Kh. S. Koshtoyants and S. S. Mogoras [4], in experiments on frogs, demonstrated that the reflex inhibition of the activity of the heart may also be removed by the action of methylene blue on the heart. It was shown in work by E. A. Kyandzhuntseva [6] that methylene blue inhibits the action of the vagus nerve on the heart of warm-blooded animals also.

A large number of facts has recently been accumulated showing the importance of sulfhydril groups in the transmission of nervous excitation, in particular in relation to the influence of the vagus nerve on the heart [3, 5, 7, 8].

Ks. S. Koshtoyants has postulated that the effect of methylene blue may be explained by the interaction between methylene blue and the sulfhydril groups. This hypothesis was based on biochemical data which indicated that methylene blue, as a hydrogen acceptor, may take part in the process of conversion of SH-groups into SS-groups. In order to verify this hypothesis experimentally, we carried out experiments to discover whether it is possible to restore the inhibiting action of the vagus nerve and also of acetylcholine on the cardiac muscle, blocked by methylene blue, by the action of substances such as SH-glutathione and hydrogen sulfide. In the present report we describe the results of these experiments.

EXPERIMENTAL METHOD

Experiments were carried out on frogs' hearts, isolated by Straub's method and perfused through the conus arteriosus. In cases where the vagus nerve was to be stimulated, the heart remained connected to the medulla oblongata through the vagosympathetic trunk; the vagus nerve center in the medulla was stimulated by means of silver electrodes. The experiments were recorded by means of a mechanical recording system and by electrocardiograms of the heart.

The action of the following substances was tested: 1) methylene blue — $2 \cdot 10^{-3}$; $2 \cdot 10^{-4}$; $2 \cdot 10^{-5}$ g/ml; 2) acetylcholine — $1 \cdot 10^{-6}$; 3) reduced glutathione — $1 \cdot 10^{-2}$ g/ml; 4) atropine — $1 \cdot 10^{-5}$; $1 \cdot 10^{-6}$ g/ml; 5) hydrogen sulfide (H_2S).

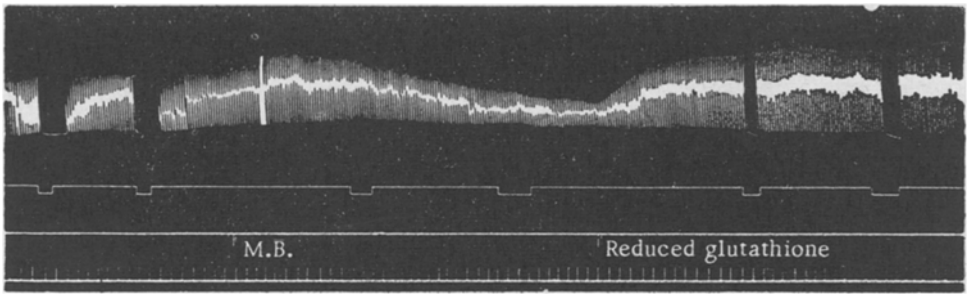

Fig. 1. Restorative action of glutathione on the effect of the vagus nerve after the action of methylene blue.
Significance of the curves (from above down); contractions of the cardiac muscle; stimulus marker; marker of the moment of injection of the substances into the heart (methylene blue $2 \cdot 10^{-4}$, reduced glutathione $1 \cdot 10^{-2}$); time marker (5 seconds).

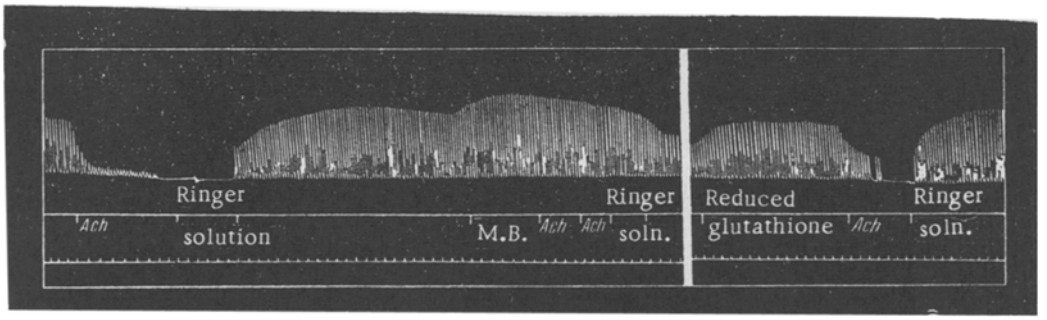

Fig. 2. Restorative action of glutathione on the effect of acetylcholine after the action of methylene blue.
Significance of the curves as in Fig. 1. Acetylcholine — $1 \cdot 10^{-6}$ (0.04 ml) methylene blue — $2 \cdot 10^{-4}$; reduced glutathione — $1 \cdot 10^{-2}$.

The solutions of methylene blue and reduced glutathione were prepared in Ringer solution, and the solutions of acetylcholine and atropine in distilled water. Ringer solution was saturated with H_2S from a Kipp's apparatus. The gas was usually passed through a tube of known diameter for 5-20 sec into 20-25 ml of Ringer solution.

The number of H_2S bubbles passing through a given volume of solution was recorded on the kymogram (H_2S — 10/25). The solutions of hydrogen sulfide and glutathione were freshly prepared on the day of the experiment; the pH of the solutions was from 7.0 to 7.5.

EXPERIMENTAL RESULTS

By investigating the action of methylene blue on the frog's heart we established that this substance, in concentrations of $2 \cdot 10^{-3}$ and $2 \cdot 10^{-4}$, blocks the action of the vagus nerve on the heart after $1\frac{1}{2}$ to $3\frac{1}{2}$ minutes.

Prolonged perfusion of the heart with Ringer solution (for 40-50 minutes) after this may lead to restoration of the effect of stimulation of the vagus nerve center. After the prolonged action of methylene blue on the heart, the inhibiting effect of the vagus nerve cannot be restored by perfusion of the heart with Ringer solution.

Comparison of the mechanographic and electrographic recordings showed that in 80% of the experiments the effect of the vagus nerve on the heart, after the action of methylene blue, is altered and often is completely

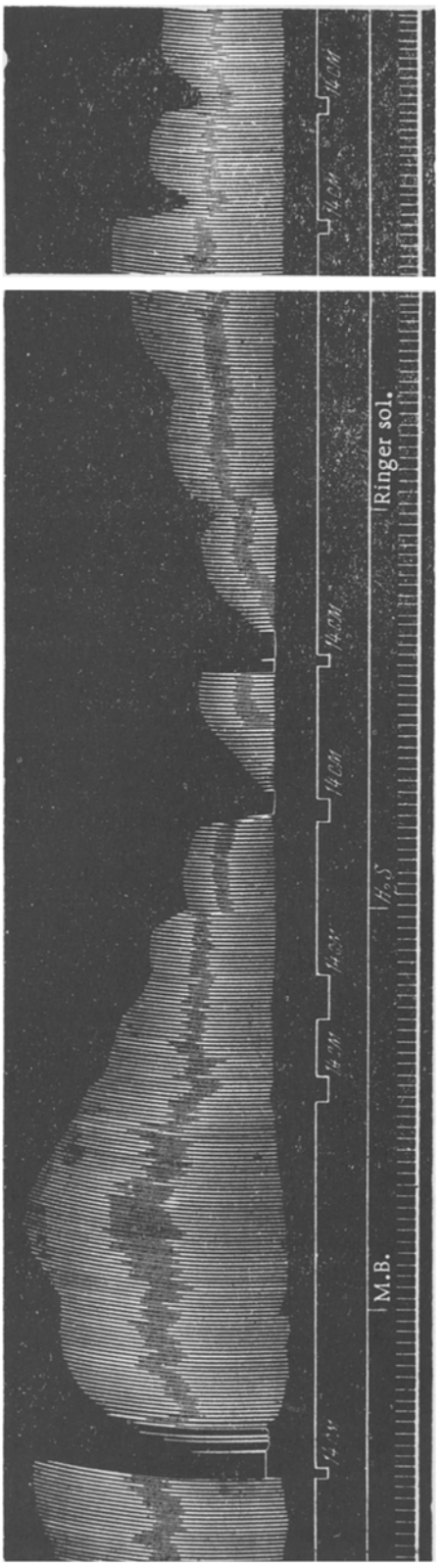

Fig. 3. Restorative action of hydrogen sulfide on the vagus nerve effect. Significance of the curves as in Fig. 1. H_2S – 10/25.

blocked; in 49% of the 80% of experiments an increase in the heart rate was observed in response to stimulation of the vagus nerve center.

As the main criteria of the condition of the heart during the experiments we examined: the change in rhythm during stimulation of the vagus nerve; the change in atrioventricular conduction and also the changes in the form of the electrocardiogram (T wave).

In relation to the findings described above, M. L. Belen'kii pointed out that methylene blue in these concentrations blocked the depressing effect of acetylcholine ($1 \cdot 10^{-6}$).

The effect of acetylcholine was not restored by prolonged perfusion of the heart with Ringer solution.

On the basis of the above-mentioned hypothesis of the possible role of SH-groups in the mechanism of the effect of methylene blue on the action of the vagus nerve (and of acetylcholine), we tried experimentally to restore this action (after the prolonged action of methylene blue) by means of SH-glutathione and H_2S. In normal conditions neither glutathione nor H_2S (in the concentrations which we used) caused any perceptoble change in the rhythmic activity of the heart. Occasionally a slight increase in the amplitude of the cardiac contractions may be observed, which rapidly returned to normal; hydrogen sulfide rarely caused a slight slowing of the rate. The inhibitory effect of stimulation of the vagus nerve was hardly affected by these substances.

After introduction of methylene blue solution into the heart phasic changes were observed: at first an increase in the amplitude of the cardiac contractions, strengthening of the cardiac action and then a decrease in the amplitude of the contractions. After the methylene blue had blocked the action of the vagus nerve and of acetylcholine on the heart, the solution of methylene blue was replaced by a solution of reduced glutathione. As a rule the glutathione caused first normalization of the strength and rate of the cardiac contractions, and secondly restoration of the action of the vagus nerve and acetylcholine blocked by the methylene blue (Figs. 1 and 2).

The electrocardiograph recordings confirmed that acetylcholine causes considerable changes in the electrocardiogram: in our experiments these were most clearly shown in the shape of the T wave and also by the shortening and almost complete disappearance of the S-T interval. Under the action of methylene blue the T wave was the most affected (it was flattened, sometimes inverted); in addition there was a significant lengthening of the S-T interval and a diminution of the

R wave. Against a background of methylene blue, acetylcholine caused none of the changes in the electrocardiogram that are characteristic of the action of this drug. On the addition of reduced glutathione (after the action of methylene blue) the electrocardiogram gradually returned to normal (the S-T interval diminished, the R wave and more particularly the T wave voltage increased) and at the same time, against this new background created by the glutathione, addition of acetylcholine caused its characteristic, normal changes in the electrocardiogram which did not appear when acetylcholine was added in conjunction with methylene blue.

Our experiments showed that hydrogen sulfide and reduced glutathione equally abolish the effect of methylene blue and restore the action of the vagus nerve and of acetylcholine on the heart (Fig. 3). When the action of hydrogen sulfide is brief, its effect is reversible in character: addition of methylene blue solution caused the action of the vagus nerve to be blocked; it was restored by perfusion of the heart with Ringer solution saturated with H_2S, and when this was replaced by fresh Ringer solution, marked weakening or disappearance of the vagus effect was again observed. Such a competitive relationship between methylene blue and hydrogen sulfide is probably connected with a transient action of the latter, which can only permit partial and insignificant restoration of SH-groups.

A special series of experiments was devoted to the comparative analysis of the action of methylene blue and atropine (as agents inhibiting the action of the vagus nerve on the heart), in order to discover the role of SH-groups in either case. The experiments showed that whereas after the action of the vagus nerve has been blocked by methylene blue it can be restored by perfusion of the heart with Ringer solution containing hydrogen sulfide, it cannot be restored in the same way after being blocked by atropine.

SUMMARY

The disturbance of the process of "transmission" of the nervous excitation may be caused by chemical agents blocking one or another link of the metabolic process. Methylene blue is one of the substances blocking the transmission of the nervous excitation from the vagus nerve on the heart. Numerous facts point to the important role played by SH groups in the transmission of the nervous excitation. A suggestion was made on the possibility of the reestablishment of the inhibitory effect of the vagus nerve and acetylcholine on frog's heart previously blocked by methylene blue substances containing SH groups (SH-glutathione and by H_2S).

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