EXCITATORY EFFECT OF ACETYLCHOLINE ON THE REFLEXOGENIC ZONE OF THE EPICARDIUM AND PERICARDIUM IN VAGOTOMIZED ANIMALS

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During stimulation of the spinal afferent system of the epicardium and pericardium with acetylcholine in concentrations of 0.001-1000 μg/ml, any concentrations above threshold produces pressor effects exclusively. These can be divided into two groups: interoceptive proper, and nociceptive. By recording the blood pressure it was shown that the first group of reflexes arises in response to concentrations of 0.001-0.5 μg/ml, and the second group, i.e., the nociceptive reflexes, to concentrations of 1-10 μg/ml. The graph of acetylcholine concentration versus amplitude of reflexes consists of three limbs, and in principle it resembles the graph for the reflexogenic zone of the small intestine obtained by Khavutin and co-workers (1964). At the same time, threshold concentrations of acetylcholine for the cardiac reflexogenic zone are lower than for receptors of the small intestine.

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Injection of acetylcholine and other chemical stimulants into blood vessels of the small intestine leads to the development of two types of pressor reflexes: interoceptive proper, and nociceptive [1-3,5,6]. The latter arise as the result of direct excitation of afferent fibers [2, 3, 5, 6]. Threshold concentrations of acetylcholine for the development of interoceptive and nociceptive pressor reflexes evoked by stimulation of the receptor zone of the small intestine are 0.1 and 10 μg/ml, respectively.

The object of the present investigation was to determine whether the principles discovered during the study of acetylcholine action on the reflexogenic zone of the small intestine extend also to the spinal afferent system of the epicardium and pericardium.

EXPERIMENTAL METHOD

A "window" measuring 4×6 cm was formed in the chest wall opposite the heart in 24 cats anesthetized with urethane (1 g/kg, intravenously), and maintained on artificial respiration. The pericardial cavity was opened and the edge of the parietal layer of pericardium carefully sutured to the borders of the "window". In this way the thoracic cavity was shut off by the pericardial membrane and the heart remained outside [9]. The vagus and aortic nerves (and sometimes also the phrenic nerves) were divided so that impulses arising during the action of acetylcholine on the membranes of the heart could reach the brain only along afferent fibers of spinal origin.

For stimulation, acetylcholine was applied in concentrations of between 10^{-9} and 10^{-3} g/ml, and this was subsequently washed out with 50-70 ml of warm Ringer's solution. Depending on the conditions of stimulation the experiments were divided into two series. In series 1 (17 experiments), the membranes of the heart were irrigated with 1 ml of acetylcholine solution (38°), or cotton wool swabs, soaked in this solution, were applied to the epicardium. The intervals between stimulation were 10 min. In 7 experiments of series II these intervals were increased to 20-30 min.

To prevent the action of acetylcholine on the heart through its parasympathetic elements, and also to prevent the vasodilator effect after absorption from the surface of the heart, in all experiments atropine

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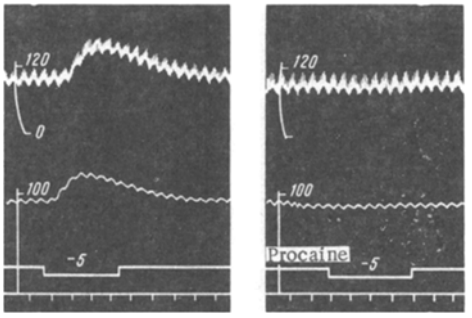

Fig. 1. Disappearance of blood pressure response to stimulation of cardiac membranes with acetylcholine after application of procaine. From top to bottom: arterial pressure recorded by spring and mercury manometers, marker of stimulation (numbers give acetylcholine concentration in g/ml), time marker 5 sec.

(0.5-1 mg/kg) was injected intravenously. The pressure in the femoral artery was recorded by a U-type mercury and a spring manometer.

EXPERIMENTAL RESULTS AND DISCUSSION

Application of acetylcholine to the epicardium and pericardium of animals with divided vagus and aortic nerves invariably produced pressor responses only. Division of the phrenic nerves caused virtually no change in the amplitude of the reflexes. The elevation of the arterial pressure was reflex in origin, for it was abolished by irrigation of the epicardium and pericardium with 2% procaine solution (Fig. 1). The curves recorded by the spring manometer (Fig. 2) indicate a link between the pressor reflexes and elevation of the diastolic pressure, and hence the predominant role of the vascular component in the mechanisms of these reflexes.

In 9 of 17 experiments, acetylcholine concentrations adequate to reach the threshold for generation of interoceptive reflexes were 0.01 μg/ml, in 5 experiments they were 0.1 μg/ml, and in 2 experiments 0.5 μg/ml. In one of the three experiments in which the action of acetylcholine was also tested in a concentration of 0.001 μg/ml, a threshold reflex appeared.

An increase in the acetylcholine concentration from 0.01 to 0.5 μg/ml was accompanied by a comparatively small increase in amplitude of the reflexes: from 2.6 ± 0.7 to 9.2 ± 0.8 mm Hg. However, a further increase in concentration, even if only twofold, (to 1 μg/ml), led to the development of disproportionately larger reflexes, with a mean amplitude of 18.5 ± 1.5 mm Hg. As a result, an inflection was formed on the concentration–effect curve, and a second limb appeared. The point of inflection of the curve in 13 of the 17 experiments corresponded to a concentration of 1 μg/ml, and in three experiments to 10 μg/ml. In one experiment the second limb was ill defined. On the average (of 17 experiments) the reflexes reached their maximum (25.5 ± 1.5 mm Hg) at a concentration of 100 μg/ml.

Since in the experiments of series I, the reflexes, especially interoceptive, were relatively small, and in three experiments these reflexes were absent, in the experiments of series II the interval between stimulation was lengthened. This lead to an increase in amplitude of the reflexes (Fig. 3) and to a steeper jump in the critical region. In 5 experiments the action of acetylcholine was tested in a concentration of 0.001 μg/ml, and in 7 experiments in a concentration of 0.01 μg/ml. Threshold reflexes at these concentrations appeared in 3 and 4 experiments, respectively. By analogy with the receptor zone of the small intestine [1, 4], reflexes in the region of the second limb of the curve must be regarded as nocicpetive. As a rule they were accompanied by movements of the forelimbs, the shoulder girdle, and head. The threshold concentration for nociceptive effects was 1 μg/ml in 5 experiments and 0.5 μg/ml in 2 experiments.

The shape of the curve of concentration versus amplitude of pressor reflexes was the same as in experiments in which the cardiac membranes were stimulated with potassium ions [7]. Similar curves have been described for stimulation of the reflexogenic zone of the small intestine [1, 4]. This similarity is presumably due to the fact that the cardiac membranes and intestinal tissues are innervated by afferent fibers with identical functional properties.

In the present experiments the minimum concentration of acetylcholine at which nociceptive reflexes appeared was lower than the concentration determined by other investigators.

The threshold of nociceptive reactions for the reflexogenic zone of the small intestine was 10-50 μg/ml [1, 4], and for injection of acetylcholine into the blood stream it was 250-500 μg/ml [10]; while the threshold of pain sensation in man (by application of acetylcholine to the base of an opened blister) is

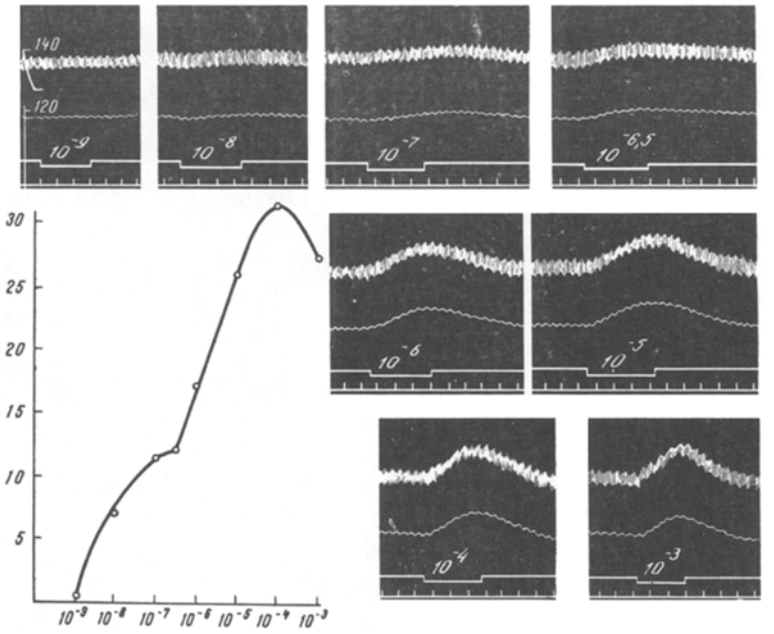

Fig. 2. Relationship between reflex elevation of arterial pressure and concentration of acetylcholine stimulating epicardium and pericardium. Legend to kymogram as in Fig. 1. For graph: ordinate, amplitude of reflexes (in mm Hg), abscissa, acetylcholine concentration (in g/ml).

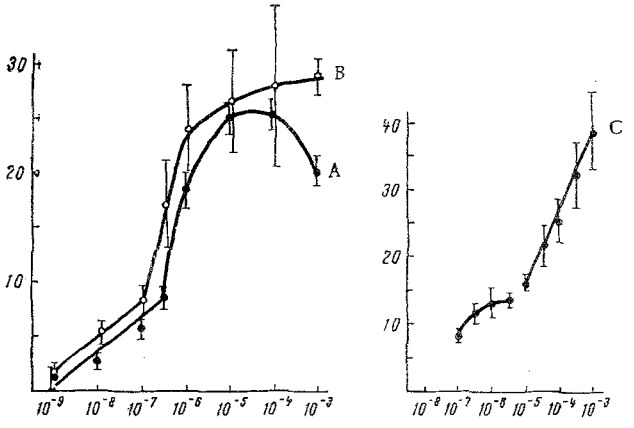

Fig. 3. Comparison of mean amplitudes of pressor reflexes evoked by acetylcholine stimulation of reflexogenic zones of epicardium and pericardium (experiments of series I and II, graphs A and B, respectively), and of small intestine (C). Legend as for graph in Fig. 2.

reached in a concentration of 10-50 μg/ml [8]. One of the reasons of the lower threshold of nociceptive effects when acetylcholine is applied to the cardiac membranes could be that it did not come into contact with the blood, and was not therefore broken down by the cholinesterase of the blood. Another important factor is the different distances of receptor zones from the spinal cord. According to Khayutin's hypothesis [6], nociceptive responses are responses to synchronized volleys of impulses in microbundles of nonmedullated afferent fibers. From this point of view, a decrease in the threshold concentration of acetylcholine can be attributed to a lower degree of desynchronization of afferent discharge on account of the closer position of the reflexogenic zones of the heart to the spinal cord compared with that of the small intestine.

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