ACTION OF MICROELECTROPHORETIC INJECTION OF MEDIATORS ON SINGLE CELLS OF THE ISOLATED GUINEA PIG ATRIUM

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Two-core coaxial microelectrodes were used to study the action of microphoretic injections of adrenalin, histamine, and acetylcholine (2 M solutions) on electrical activity of single fibers of the isolated right atrium, recorded intracellularly. Adrenalin caused an increase in the frequency of spontaneous action potentials in fibers of pacemaker type, with a latent period of about 5 sec, but did not modify the form and frequency of spontaneous action potentials of contractile fibers. Application of histamine to pacemakers and contractile fibers did not affect the character of the electrical activity of these cells. Application of acetylcholine to contractile fibers shortened the repolarization phase of spontaneous action potentials with a latent period of 3.94 ± 0.34 sec. Preliminary addition of adrenalin (1 μg/ml) to the atria did not change the duration of the latent period of the acetylcholine effect on contractile fibers. Atropine (0.1, 1, and 2 μg/ml) lengthened the latent period of the acetylcholine effect on atrial contractile fibers.

The action of excitatory and inhibitory mediators on electrical activity of single cells of the isolated atrium in animals has so far been studied by perfusion of a heart preparation with these substances. Clearly, under these experimental conditions, it is difficult to identify precisely the point of application of the drug on particular types of cells or to assess what changes in electrical activity of particular types of cells are the result of the primary action of the drug on these cells and which are the indirect result of its action on other elements of the heart preparation [5].

This paper describes the results of a study of the action of adrenalin, histamine, and acetylcholine, injected microelectrophoretically, on single cells (pacemakers and contractile fibers) of the atrium, with simultaneous recording of electrical activity of these cells by means of two-channel microelectrodes.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated atria of 15 male guinea pigs weighing 250-300 g. The method of preparation of the specimen and the experimental technique were described previously [1]. Two-core coaxial microelectrodes [3] were used. The inner microelectrode, filled with 3 M KCl (10-50 MΩ) was used for intracellular recording of the cell potentials. The tip of the outer microelectrode, about 5-10 μ in diameter, was separated from the tip of the inner microelectrode by a distance of 30-50 μ. The outer microelectrode, filled with a solution of the corresponding drug, was used for microelectrophoretic injection of the test substances near to the cell membrane. The drugs were injected as cations. To diminish diffusion of the drugs in the period between injections, a steady direct current was passed in the opposite direction through the outer electrode. The theoretical circuit used for injection of the drugs is illustrated in Fig. 1.

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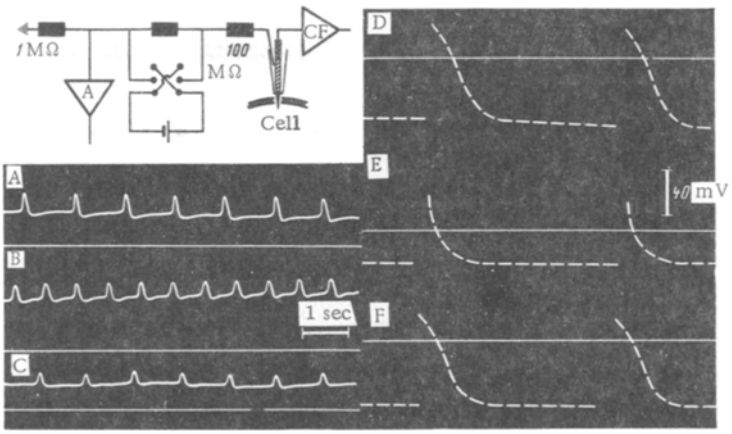

Fig. 1. Action of microinjection of adrenalin and acetylcholine on electrical activity of single cells in the right atrium. A) Initial spontaneous activity of pacemaker; B) during injection of adrenalin by current of about 300 pA; C) 20 sec after switching off injecting current; D) initial spontaneous activity of contractile fiber; E) during injection of acetylcholine by current of about 100 pA; F) 15 sec after stopping injecting current. Time marker for D–F, 20 msec; CF) cathode follower; A) dc amplifier.

EXPERIMENTAL RESULTS AND DISCUSSION

The action of adrenalin hydrochloride, injected from a 2 M solution (pH 3.0), on electrical activity of the right atrial pacemaker is illustrated in Fig. 1A-C. The initial spontaneous activity consists of characteristic action potentials for this type of fiber, generated after diastolic depolarization reaches its threshold value (Fig. 1A). During injection of adrenalin by a current of about 300 pA, a definite increase in frequency of action potentials was observed (Fig. 1B), returning to the initial level after stopping the injecting current (Fig. 1C). The latent period of increase in frequency of action potentials at the moment of adrenalin injection was about 5 sec.

Microelectrophoretic injection of adrenalin onto contractile atrial fibers with a current of up to 500 pA, and duration of up to 40 sec, according to results obtained with 12 cells, did not lead to any appreciable changes in the frequency and form of spontaneous action potentials of these types of cells.

Microelectrophoretic injection of histamine dihydrochloride (2 M solution) by a current of up to 500 pA and duration up to 30 sec caused no changes in spontaneous activity of the pacemaker (on 8 cells) or contractile fibers (10 cells) of the right atrium.

The initial spontaneous electrical activity of a contractile right atrial fiber is illustrated in Fig. 1D. The membrane potential between individual action potentials is steady, the depolarization phase is relatively fast, and the repolarization phase is slow. During injection of acetylcholine chloride (2 M solution) by a current of about 100 pA, the repolarization phase is considerably shortened and the amplitude of the action potentials reduced, with no change in their frequency (Fig. 1E). Stopping the injecting current was accompanied by restoration of the original shape of the action potentials (Fig. 1F). The action of acetylcholine on contractile fibers frequently was apparent only when the holding current was switched off. The latent period of the acetylcholine effect on action potentials of contractile atrial fibers, according to results obtained on 22 cells, was 3.94 ± 0.34 sec (from 1.6 to 7.1 sec).

A special series of experiments was carried out to study the action of injected acetylcholine on the same contractile cells before and after addition of adrenalin to the atrial preparation in a concentration of 1 μg/ml, yielding the maximum effect assessed as an increase in frequency of spontaneous action potentials. Against the background of adrenalin, the action of the injected acetylcholine was tested at a time of maximal increase in frequency of the spontaneous action potentials. In each case the injecting current was constant in strength. The magnitude of the acetylcholine effect on the shape of the action potentials was assessed from its latent period. The latent period of the acetylcholine effect before addition of adrenalin

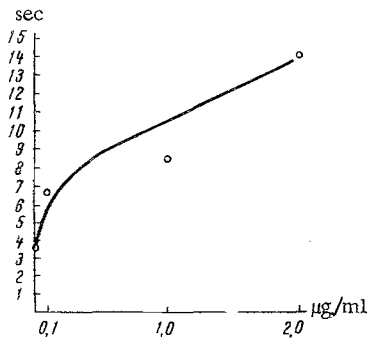

Fig. 2. Effect of addition of atropine to atria on latent period of effect of acetylcholine microinjection. Abscissa, atropine concentration (in μg/ml); ordinate, latent period (in sec).

to the atria was 3.57 ± 0.53 sec (from 1.6 to 6.2 sec), and on the same cells against the background of adrenalin action it was 3.38 ± 0.38 sec (from 1.6 to 5 sec, $P > 0.05$).

Addition of atropine to the atrial preparation in concentrations of 0.1, 1, and 2 mg/ml lengthened the latent period of the acetylcholine effect (Fig. 2).

In the first place, the results of these experiments give direct evidence of the action of adrenalin on pacemakers of the isolated guinea pig atrium. At the same time, no appreciable changes in the frequency or shape of spontaneous action potentials of the contractile fibers were found in this investigation in response to microelectrophoretic application of adrenalin to them.

Results of the study of adrenalin action on electrical activity of contractile fibers of the mammalian atrium during perfusion of a whole preparation by this drug are contradictory. In some cases no changes whatever were found in the shape of the action potentials, while in others, a slight increase in duration of the repolarization phase was recorded [4, 5]. The absence of changes in shape of action potentials of contractile fibers during electrophoretic application of adrenalin to them indicates that lengthening of the repolarization phase, sometimes observed after application of adrenalin to the atrium, is not necessarily the result of the primary action of this mediator on the contractile fibers of the atrium.

The absence of change in frequency of action potentials of pacemaker fibers or in the shape of action potentials of contractile fibers observed in this investigation during microelectrophoretic application of histamine to them confirms the hypothesis that the positive chronotropic action of histamine on the heart preparation is not due to the direct action of histamine on myocardial cells, but is perhaps the result of excitation of postganglionic sympathetic nerves [7].

The direct action of microelectrophoretically injected acetylcholine on the contractile fibers of the atrium consists of a considerable shortening of the repolarization phase of the spontaneous potentials with no change in their frequency. This effect of acetylcholine on the shape of the action potentials of contractile fibers is analogous to the action of this mediator when added to the whole atrial preparation [4, 6].

Some authors [2] have postulated that adrenergic and cholinergic receptors of heart muscle are identical, and in support of this they adduce, in particular, the fact that the acetylcholine effect, assessed from the contractile response of the myocardium, can be reduced by preliminary addition of adrenalin to the heart preparation. According to the present results, adrenalin in concentrations producing a maximum increase in frequency of action potentials did not modify the duration of the latent period of action of acetylcholine when injected onto contractile fibers.

If this decrease in acetylcholine response, as reflected by the mechanical response of the heart muscle against the background of adrenalin action, is in fact due to interaction of both mediators on the same receptor protein molecule [2], then in that case inhibition of another index of acetylcholine action on the cell membrane would also be expected against the background of adrenalin, namely the increase in latent period of action of injected acetylcholine on the electrical activity of the myocardial cells. Lengthening of the latent period of the acetylcholine effect was clearly observed when cholinergic receptor structures were blocked by atropine.

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