ducts (n = 14) and retrograde injection of saliva into the ducts (n = 17), leading to vasodilatation of the gland [1] and accompanied in the present experiments by a reduction of electrical resistance, did not change the electrical potential when recorded by the two methods.

Considering previous data [8-10], these findings indicate the glandular origin of the electrical responses. Much greater hyperpolarization of the apical membranes than of the basal membranes must be assumed in the acinar cells of the parotid gland. Should the acini be oriented away from the hilus of the gland, summation of the set of dipole-acini into one equivalent source would take place, the distance from the gland to the surface of the body would be much less than the size of the source, and the potential gradient would be absent. Such a model, in agreement with the experimental data, explains the electrical response on the surface of the body and allows it to be used for investigations of glandular tissue.

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EFFECT OF ACETYLCHOLINE ON DISCHARGE FREQUENCY AND SHAPE OF ACTION POTENTIALS OF PACEMAKER CELLS

G. I. Bochkina, G. S. Sukhova, and M. G. Udel'nov

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The action of exogenous acetylcholine on the isolated pacemaker of the frog heart was studied. Activity of single cells and the total activity of the preparation were recorded. Exogenous acetylcholine had not only an inhibitory, but also an accelerating effect on the rhythm of discharge of the pacemaker cells. As a rule an accelerating action was observed when relatively low concentrations of acetylcholine were used. An increase in the rate of rise of slow diastolic depolarization during the development of parasympathetic acceleration is evidence of the active mechanism of this process. The difference in the effects of acetylcholine was probably due to differences in the action of large and small concentrations of the drug on transmembrane ionic currents.

KEY WORDS: acetylcholine; pacemaker cells; acceleration of rhythm; inhibition of rhythm.

Investigations have shown that the parasympathetic innervation of the heart can cause opposite effects on the heart beat [4, 5]. It has recently been found that impulses acting through cholinergic mediator can either delay or accelerate the development of slow diastolic depolarization (SDD) and, at the same time, delay or accelerate the discharge frequency of pacemaker cells [1, 3]. Similar studies of the action of exogenous acetylcholine (ACh) on the development of SDD and on the rhythm of the pacemaker cells have not been under-

Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow State University.

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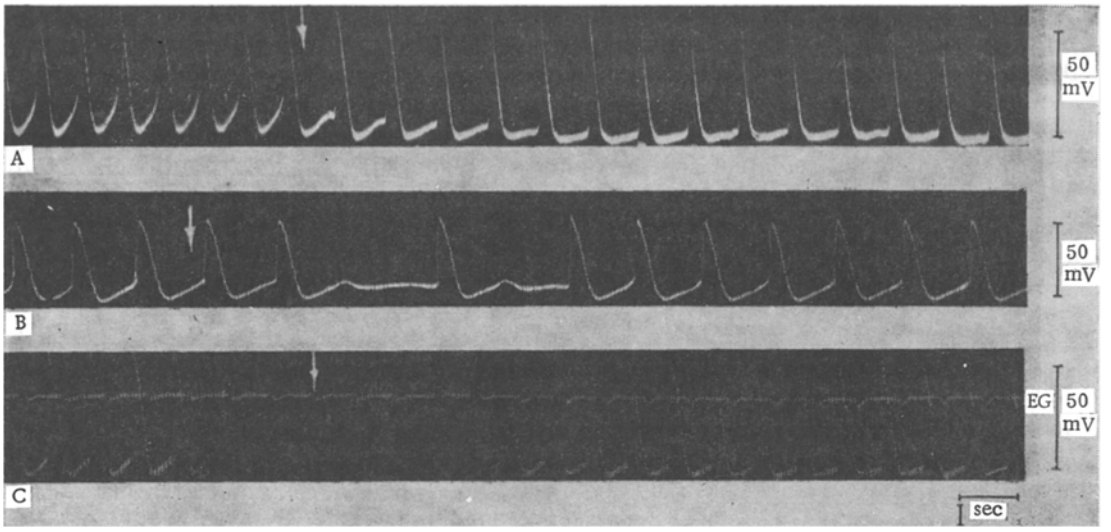

Fig. 1. Different types of inhibitory action of ACh on pacemaker cells. A) Inhibition of rhythm of pacemaker cell discharges during action of ACh in concentration of $1 \cdot 10^{-10}$ g/ml against the background of hyperpolarization, decrease in rate of rise of SDD, and shortening of duration of AP; B) deeper inhibition of rhythm taking place during very small changes in shape of AP; C) decrease in rate of rise of SDD and shortening of duration of AP in absence of chronotropic effect. Here and in Fig. 2, EG denotes electrical activity of isolated pacemaker. Arrow marks time of application of ACh.

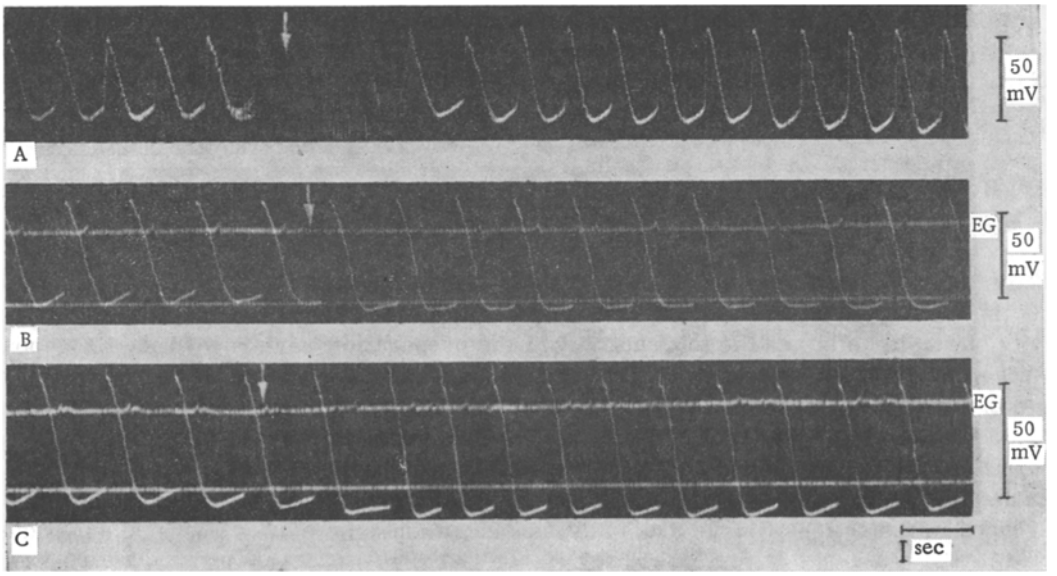

Fig. 2. Acceleration of rhythm of pacemaker cell discharge by the action of ACh. A) Increase in rhythm from 75 to 84 beats/min against background of more rapid development of SDD, shortening of duration of AP, and hyperpolarization; B) acceleration of discharges from 58 to 67 beats/min developing during considerable decrease in rate of rise of SDD, shortening of duration of AP, and reduction in hyperpolarization; over-all rhythm of discharge of preparation shows parallel change with rhythm of discharges from single cell; C) more marked acceleration of rhythm (from 57 to 75 beats/min) accompanying small decrease in rate of rise of SDD, shortening of duration of AP, and reduction in hyperpolarization of membrane; total rhythm of discharge of preparation became irregular after application of ACh.

taken. The explanation of this problem is important also because the view has hitherto been widely accepted that exogenous ACh can have only an inhibitory action on the cardiac pacemaker.

The object of the present investigation was to verify how consistently negative chronotropic effects are observed as a result of the action of exogenous ACh on pacemaker cells.

EXPERIMENTAL METHOD

Experiments were carried out on a preparation of the isolated sinus venosus of the frog heart. Electrical activity of the pacemaker cells was recorded by glass floating microelectrodes with a tip under 0.5 μ in diameter, filled with 3M KCl solution. In most experiments parallel recordings were made of the total electrical activity of the preparation by means of wick macroelectrodes. Both processes were recorded on a loop oscillograph. ACh was applied to the surface of the whole pacemaker by means of a thin pipette. Acetylcholine was used in concentrations of between $1 \cdot 10^{-9}$ and $1 \cdot 10^{-11}$ g/ml.

EXPERIMENTAL RESULTS AND DISCUSSION

The experiments showed that exogenous ACh gives rise to different changes in the electrical activity of the pacemaker cells.

In most experiments the rate of rise of SDD was reduced, the polarization of the membrane was increased, and the duration of the action potentials (APs) was shortened by ACh. These bioelectrical changes were accompanied by slowing of the rhythm (Fig. 1A). These effects are in agreement with the widely known data [9, 10, 13]. However, in different cells of the same preparation the above changes in shape of the AP, which are usually regarded as inhibitory, could be observed in different combinations. Clear correlation likewise was not observed between the depth of the negative chronotropic effect and the degree of the changes in shape of the AP. For instance, an example of the inhibitory effect of ACh on the discharge frequency of a pacemaker cell is illustrated in Fig. 1B; it was not accompanied by any decrease in the rate of development of SDD or any appreciable change in the other phases of AP. In other cases ACh could significantly change the shape of the AP without changing the discharge rhythm of the cell (Fig. 1C).

Together with the effects of ACh in the concentrations specified described above, in some cases the discharge frequency of the pacemaker cells was increased. Sometimes acceleration developed against the background of an adequate increase in the rate of rise of SDD (Fig. 2A). At the same time hyperpolarization was observed and the duration of the APs was shortened, both of which are regarded as inhibitory effects. In other cases acceleration of the discharge of the pacemaker cell was accompanied by a decrease in the rate of rise of SDD, a decrease in hyperpolarization of the membrane, and shortening of the duration of AP. The over-all rhythm of the preparation changed parallel with the change in the discharge frequency of the recorded cell (Fig. 2B). A case when application of ACh caused an even more marked increase in frequency than in the first two cases, accompanied by a small decrease in the rate of rise of SDD, is illustrated in Fig. 2C. Characteristically in this case the over-all rhythm of the preparation became irregular after application of ACh, with clearly defined slow cycles.

Besides slowing of the rhythm, in some cases ACh was thus able to cause acceleration of the rhythm of the pacemaker cell discharges. The positive chronotropic effect under these circumstances was not always associated with the more rapid development of SDD. Cases when acceleration of the discharge was combined with a decrease in the rate of rise of SDD could probably be explained not only by the action of ACh on the particular cell, but also by a change in interaction between structures of the pacemaker in which acceleration of the rhythm developed against the background of adequate changes in the shape of AP. Investigations have shown that AP and the rhythm of pacemaker cells depend to a large extent on intercellular interaction [6].

Positive chronotropic effects and changes in electrical activity of the pacemaker cells associated with them, produced by the action of ACh, were abolished by atropine, demonstrating the cholinergic nature of these effects. Since hyperpolarization and the more rapid development of phases of repolarization could be accompanied not only by slowing, but also by acceleration of the discharge of the pacemaker cells, it is reasonable to consider that these changes in bioelectrical activity were an indication not of an inhibitory effect, but of a cholinergic effect that may be two-directional.

Since the effect depends not only on the nature of the pulsed mediator action, but also on the properties of the reacting cells, it can tentatively be suggested that the variation of the effects produced by cholinergic stimulation is due to differences in the reactive properties of the pacemaker cells. The cells may differ in their sensitivity to ACh, in their functional state and also, perhaps, in certain genetically determined features.

Some workers consider that differences in the response of pacemaker cells are structurally and functionally consolidated [12]. Other investigations have shown that the reactive properties may vary with time and also under the influence of certain conditions [6]. The intimate mechanisms of the accelerating effect of ACh has not yet been explained. It is considered that ACh increases the permeability of the cell membrane for potassium ions, and this is responsible for the inhibitory effects of this mediator. Work has recently been published, however, to show that ACh may change potassium permeability in two directions. Experiments with radioactive potassium showed that ACh, in low concentrations, does not increase but reduces the permeability of the membrane for potassium [11]. This fact could explain the positive chronotropic effect. The difference in the action of high and low concentrations of ACh on transmembrane ionic currents has been shown for the trabeculae of the atrium [8] and pacemaker cells [7]. The ability of ACh to cause opposite changes depending on concentration has also been shown on cells of the intramural nervous system of the heart [2].

In the present experiments acceleration of the discharges of the pacemaker cells was observed as a rule in response to relatively low ACh concentrations ($1 \cdot 10^{-11}$ g/ml). When the same concentration of ACh was used, different cells could respond differently: some by inhibition, others by acceleration of the discharge. Probably these opposite effects were due to differences in the sensitivity of the cells to ACh. It can tentatively be suggested that cells responding to a given concentration of ACh by inhibition are more sensitive to it and that the concentration acting was sufficiently high for them, whereas cells responding by acceleration have lowered sensitivity to this mediator.

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