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PHYSIOLOGY

Acetylcholine-Induced Suppression of Electric Activity of Working Myocardium of the Cod Atrium

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Parasympathetic regulation is very important for normal work of heart of vertebrates. The neurotransmitter of postganglionic parasympathetic neurons acetylcholine (ACh) acts on the myocardium via muscarinic receptors and induces a decrease in both the duration of the action potential (AP) and contraction force, as well as many other effects [1, 2]. The action of ACh on the atrial myocardium of the majority of vertebrates is considerably stronger than its action on the ventricles [1–3]. In a working atrial myocardium of mammals, ACh induces considerable shortening of the AP but practically does not influence the AP amplitude [3]. Quite a different situation was observed in the frog atrium [4, 5]. Different parts of a working myocardium of the frog atrium have different responses to high concentrations of ACh or stimulation of parasympathetic postganglionic nerves. A strong shortening of the AP with a small decrease in the amplitude was observed in some parts of the atrium, whereas in the other parts ACh induces a gradual decrease in the AP amplitude until a complete suppression of electric activity. As a result, the frog atrium stimulated by ACh has inexcitable parts, which may initiate circulation of re-entry excitation [4, 5]. Despite the fact that ACh never induces the appearance of inexcitable zones in the working myocardium of mammalian atria, a similar situation was observed in the sinus node of mammals [6], where the arrhythmogenesis may also be caused with the involvement of inexcitable parts. Hence, the phenomenon of ACh-induced unnexcitability of the atrial myocardium is of great theoretical and practical interest.

Mammals and amphibian strongly differ in the atrial response to ACh; hence, the question is whether this difference between the lower (fishes and amphibians) and higher vertebrates is universal. To answer this question, we studied the effect of ACh on the parameters of electric activity in the working atrial myocardium of White Sea coastal cod (*Gadus morhua marisalbi*).

METHODS

The work was performed at the White Sea Biological Station of Moscow State University.

All experiments were performed in August. We used 11 small (100–200 g) specimens of cod (*Gadus morhua marisalbi*). The cod was captured and kept in the net corf put into the sea until the experiment. The cods were decapitated, and the heart was isolated and placed into physiological saline for sea fishes, which contained (in mM): NaCl, 150; KCl, 5.14; $NaH_2PO_4 \times 2H_2O$, 1; $NaHCO_3$, 25; $MgSO_4 \times 7H_2O$, 1.8; $CaCl_2 \times 2H_2O$, 1.25; and glucose, 5 [7]. The saline was bubbled with carbogen (a gas mixture of 95% of O_2 and 5% of CO_2), the saline temperature was maintained at 10°C by means of a Haake F3 thermostat (Germany). It was shown that this temperature is physiologically normal for cod during this time of the year [8].

We dissected the atrium and placed it, the inner side up, into a perfusion chamber. In nine experiments, the pacemaker located, in the cod, in the sinoatrial node ring [8] was eliminated, and electrodes applied onto the surface of the preparation were used to perform stimulation with a frequency of 1 Hz. In two experiments the preparations have their native pacemakers. The rate of chamber perfusion with oxygenated saline was 10 ml/min. Under these conditions, the preparation may normally function for many hours without any changes in its work.

To record the APs of the cod myocardium, we used the standard microelectrode method of intracellular recording. The signal was recorded using a computer and the L-graph software (L-card, Russia). After 1-h perfusion with normal saline, we added a solution that contained 0.5 μM ACh (Sigma) for 5 min; then, for 15 min the preparation was washed by the normal solution; after this, it was treated with a solution with a higher concentration of ACh. In each preparation, ACh was used at concentrations of 0.5, 1, 5, 10, and 50 μM. The recordings of the electric activity were made at the

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The main parameters of AP before ACh application. The first group (strong ACh-induced decrease in the AP amplitude), $n = 6$. The second group (weak decrease in the amplitude), $n = 3$

Parameters	Group	
	first	second
AP amplitude, mV	99.2 ± 4.8	102.1 ± 3.5
Duration of AP 50%, ms	208.9 ± 18.7	221.3 ± 12.5
Maximum rate of the AP front, V/s	17.2 ± 3.7	39.2 ± 5.1

same point of the preparation. We evaluated the AP amplitude, AP duration at the level of 50% repolarization, and ACh-induced changes in these characteristics. The significance of changes was evaluated with the use of Wilcoxon's paired test.

RESULTS

The absolute amplitude, duration, and maximum rate of the AP front are shown in the table. ACh at the concentrations used induced a dose-dependent decrease in the duration of APs in all preparations. In six out of nine preparations that were electrically stimulated, ACh also strongly decreased the amplitude of APs. In three preparations, high concentrations of ACh caused only a small decrease in the amplitude. Thus, the experiments may be subdivided into two groups according to the response to ACh. Note that these groups also differed in the initial slope of the AP front (table). It was considerably larger in the preparations where the amplitude weakly changed during ACh application.

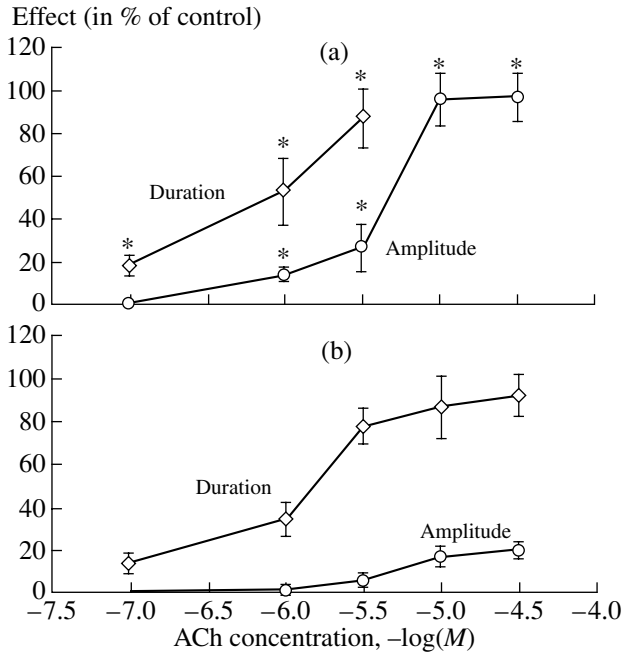

Fig. 1. ACh-induced dose-dependent decrease in the AP duration and decrease in the AP amplitude. The ordinate axis: shortening and decrease in the AP amplitude in % of control duration and amplitude of AP, respectively. (a) Group with a strong decrease in the AP amplitude during ACh application, $n = 6$. Asterisk, significant difference as compared to control, Wilcoxon test, $p < 0.05$; (b) group with a small decrease in the amplitude, $n = 3$.

Figure 1 illustrates the dose–effect relationships in the preparations that were electrically stimulated. It is seen that in the first group (Fig. 1a) high concentrations of ACh (10 and 50 μM) induced a strong decrease in the AP amplitude and, hence, the recorded small peaks are most likely to have been electrotonic potentials. Therefore, the duration of these potentials was not evaluated. Suppression of APs by 10 μM ACh is shown in Fig. 2a. In the second group, ACh caused only a small decrease in the AP amplitude, to 20.7% in the case of the maximum concentration of ACh (Fig. 1b).

In two experiments performed with preparations with intact pacemakers, we observed a similar difference in the response to ACh. In the first experiment, we observed suppression of the electric activity (Fig. 2b) in the presence of negligible inhibition of the sinus rhythm. In the second experiment, we observed a small decrease in the amplitude, which was accompanied by a decrease in the rhythm until stoppage, which lasted for 16 s and gave way to electric activity with a slowed rhythm (Fig. 2b).

DISCUSSION

In each experiment, we recorded activity only in a single point of preparation, hence, it may be hypothesized that the difference between two groups of preparations reflected the difference between ACh-induced responses in different areas of the atrium. Thus, as in the case of the frog, the cod atrium has zones that lose excitability after treatment with high concentrations of ACh, whereas the rest of the atrium is still excitable. The points where inexcitability appears were more abundant (in 7 out of 11 cases), hence, it is possible that the area occupied by inexcitable zones is larger than the area of the zones where the activity is present during the action of high concentrations of ACh. It is impossible to determine the size and shape of the inexcitable area with the use of the microelectrode method; therefore, to solve this problem, it is feasible to use optical mapping.

Note that we demonstrated gradual fading of the activity rather than its short-term loss. In the experiment with the sinus rhythm, a decrease in the AP amplitude was accompanied by only a negligible decrease in rhythm; i.e., fading of activity is not related to stoppage of the pacemaker; it is a result of the action of ACh on the working atrial myocardium. Stoppage of activity in the frog atrium during stimulation of the vagus nerve

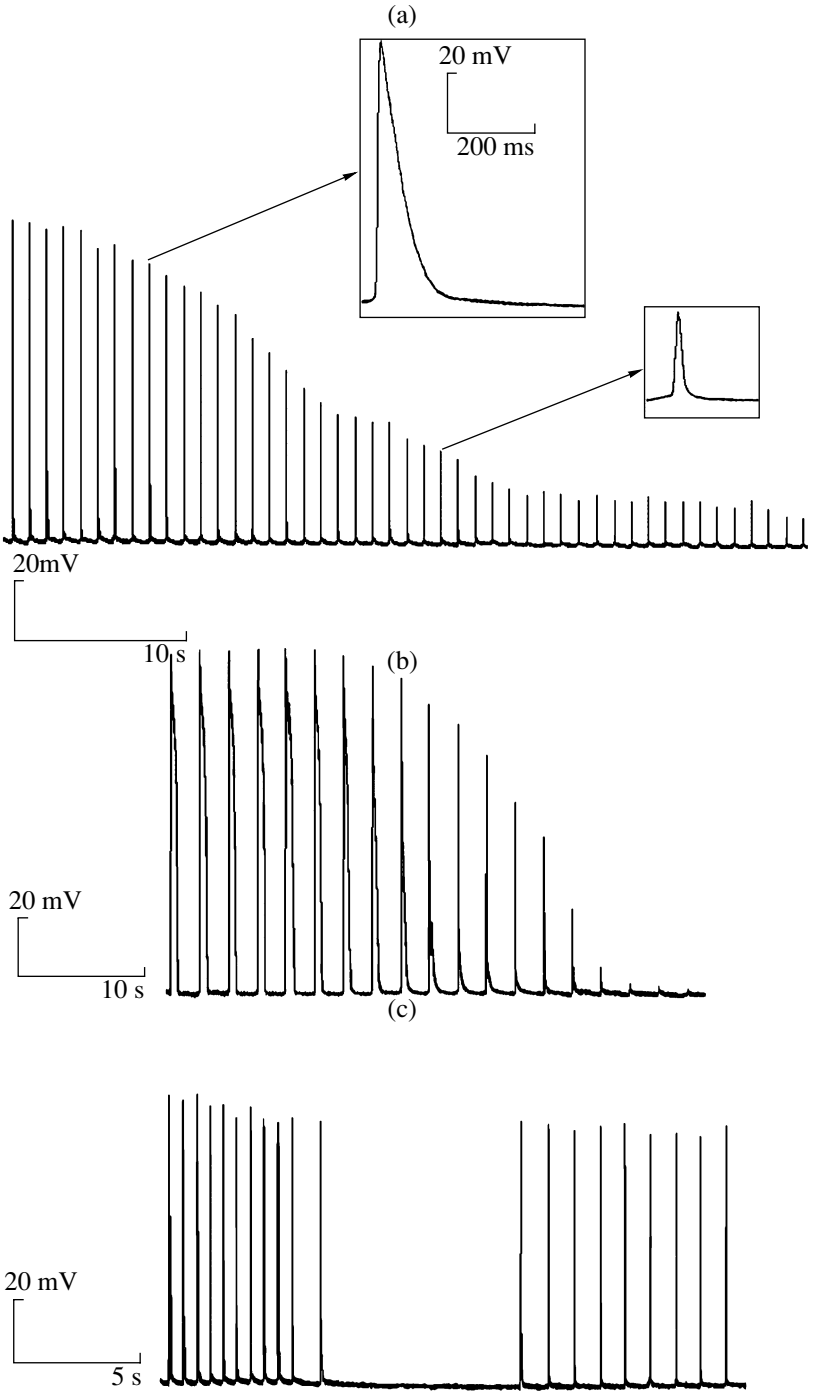

Fig. 2. Examples of original recordings. (a) Suppression of electric activity by 10 μM ACh in the preparation which worked at the imposed rhythm; (b) the same as in Fig. 1a, but in the preparation with intrinsic rhythm; (c) recovery of the activity after a short-term pause in the area of preparation where APs were not suppressed by 10 μM ACh; the preparation worked in the intrinsic rhythm.

was shown in [9]; it seems that the authors observed a phenomenon similar to the phenomenon that we found, however, they did not analyze it.

Thus, in this work, we found ACh-induced suppression of electric activity in a working myocardium of the fish atrium. Earlier indirect evidence for this phenomenon were observed [10] in isolated trout cardiomyocytes, but the authors did not discuss these data. The ionic mechanism of this phenomenon is of interest, because it has been shown that the front of APs in the fish myocardium is formed mainly due to inward sodium current [11], and the sodium current in the cardiomyocytes of higher vertebrates is not sensitive to ACh [12]. Was the current found in our study some peculiar sodium current suppressed by ACh?

What does the phenomenon described mean? On the one hand, it is clear that ACh-induced stoppage of the activity cannot occur in all atrium because in this case conducting of excitation from the pacemaker to the ventricle will stop. Therefore, in other areas that constitute the conducting pathway from the pacemaker to atrioventricular bundle the activity is not changed during ACh treatment. In the rest of area ACh may induce inhibition. On the other hand, appearance of inexcitable areas may result in the development of atrial tachyarrhythmias [4, 5]. We did not observe this phenomenon in our experiment; however, it was shown in a work [13] performed with the tilapia atrium that acetylcholine-induced arrhythmias may develop in fishes.

Thus, our data suggest that lower vertebrates differ from higher vertebrates in the ACh-induced response of the atrial myocardium.

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