Comparative Biochemistry and Physiology, Part A xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Cholinergic modulation of activation sequence in the atrial myocardium of non-mammalian vertebrates

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ARTICLE INFO

Article history:
Received 5 July 2009
Received in revised form 1 November 2009
Accepted 2 November 2009
Available online xxxx

Keywords:
Acetylcholine
Atrium
Action potential
Arrhythmia
Fish
Frog
Reptilian

ABSTRACT

Cholinergic changes of electric activity were studied in isolated atrium preparations from fishes (cod and carp), amphibians (frog) and reptilians (lizard) using the microelectrode technique and high-resolution optical mapping. Perfusion of isolated atrium with acetylcholine (10^{-6}–$5 \cdot 10^{-5}$ M) caused gradual suppression of action potential generation and, eventually, completely blocked the excitation in a part of the preparation. Other regions of atrium, situated close to the sinoatrial and atrioventricular junctions, remained excitable. Such cholinergic suppression of electric activity was observed in the atrial myocardium of frog and in both fish species, but not in reptilians. Ba^{2+} (10^{-4} M), which blocks the acetylcholine-dependent potassium current (I_{KACh}), prevented cholinergic reduction of action potential amplitude. In several preparations of frog atrium, cholinergic suppression of excitation coincided with episodes of atrial fibrillation. We conclude that the phenomenon of cholinergic suppression of electric activity is typical for atria of fishes and amphibians. It is likely to be caused by I_{KACh} activation and may be important for initiation of atrial arrhythmias.

1. Introduction

It is widely recognized that atrial fibrillation in mammals may be induced by vagal stimulation or application of the parasympathetic neurotransmitter acetylcholine (ACh) (Nadeau et al., 1970; Rosenshtraukh and Zaitsev, 1990). ACh is known to decrease action potential duration and suppress pacemaker activity in atrial tissues of lower and higher vertebrates. These effects are due mainly to the activation of outward potassium ACh-dependent currents and suppression of funny current (I_f) and inward slow Ca^{2+} current (I_{CaL}) (Harvey and Belevych, 2003). Arrhythmogenic effects of ACh are usually interpreted as a result of non-uniform shortening of the atrial refractory period that leads to an increased probability of the circus movement (Allessie et al., 1977). One of the hypotheses concerning the initiation of atrial fibrillation is based on microelectrode experiments conducted on the frog atria. Vagal stimulation provokes suppression of action potential (AP) generation in some fibers of frog atria, while in other fibers, electrical activity persists (Rozenshtraukh and Kholopov, 1975; Rosenshtraukh et al., 1989). Therefore, vagal stimulation leads to formation of inexcitable regions in the atrial myocardium and closed circular conduction pathways that provoke initiation of re-entrant tachyarrhythmias (Rosenshtraukh et al., 1989; Rosenshtraukh and Zaitsev, 1990).

In mammalian atria ACh doesn't affect the AP amplitude (APA) (Hoffman and Cranefield, 1960), although it blocks excitation in the central part of the rabbit sinoatrial node (Vinogradova et al., 1998; Fedorov et al., 2006). Fish atrial myocardium was suggested as an appropriate model for investigating the mechanisms of cholinergic re-entrant tachyarrhythmias. Lin et al. (2000) demonstrated induction of tachyarrhythmias by ACh (1–10 μM) in tilapia atrial myocardium. The incidence of tachyarrhythmias was extremely high (>50%) at 37 °C. Therefore, the tilapia provides a good alternative to mammals for the investigation of cholinergic arrhythmias. The authors proposed the non-uniform electrophysiological responses to ACh among different cell types to be the main reason of cholinergic re-entrant arrhythmias.

Thus, cholinergic effects in the atrial myocardium of different lower vertebrates are of significant interest. We have used the microelectrode technique and the novel method of high-resolution optical mapping to study changes in the AP configuration and activation sequence in isolated atrium preparations from fishes (cod and carp), amphibians (frog) and reptilians (lizard). Combined use of intracellular recordings and optical mapping allowed us to demonstrate the regional variation in excitability of the atrial myocardium in teleosts and frog in response to ACh, which can presumably lead to initiation of tachyarrhythmias. We have also investigated the ionic mechanisms of these cholinergic effects in fish and amphibian atrium.

2. Materials and methods

The experimental protocol was approved by the Bioethics Committee of Moscow State University.

Experiments with cod (*Gadus morhua* L.) were performed at the White Sea Biological Station of Moscow State University (Karelia,

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doi:10.1016/j.cbpa.2009.11.002

Russia). The fish (235 ± 55 g, $n = 22$) were kept in a cage, submerged into the sea (10 °C), before the experiment. Carp (*Cyprinus carpio* L.) of medium size (1.14 ± 0.12 kg, $n = 15$) were kept in a fresh water aquarium (17 °C). Frogs (*Rana temporaria* L.) (23.5 ± 3.0 g, $n = 26$) and lizards (*Lacerta agilis* L.) (19.5 ± 1.5 g, $n = 7$) were caught in a forest near Moscow during October. These animals were kept in a terrarium (20 °C) before the experiment.

The animals were decapitated, the chest was opened and the heart was rapidly removed and immersed in physiological solution for cod (composition (mM): NaCl 150, KCl 5.14, NaH_2PO_4 1, $NaHCO_3$ 25, $MgSO_4$ 1.8, $CaCl_2$ 1.25, glucose 5; and pH 7.55 ± 0.05) (Hoglund and Gesser, 1987), or carp (composition (mM): NaCl 127, KCl 5.1, $NaHCO_3$ 12, $MgSO_4$ 0.93, $CaCl_2$ 1.6, glucose 5.55; and pH 7.55 ± 0.05) (Coyne et al., 2000), or reptilians (composition (mM): NaCl 110, KCl 4, NaH_2PO_4 2.7, $NaHCO_3$ 23.8, $MgSO_4$ 1.3, $CaCl_2$ 2, glucose 10; and pH 7.45 ± 0.05) (Franklin and Axelsson, 1994), or standard Ringer's solution for frog (composition (mM): NaCl 111, KCl 3.8, NaH_2PO_4 0.2, $NaHCO_3$ 4.3, $CaCl_2$ 1.6, glucose 5; and pH 7.3 ± −0.1). All solutions were continuously bubbled with carbogen (95% O_2 and 5% CO_2), pH was adjusted with 1 N NaOH and 1 N HCl.

After isolation the preparations were placed in the perfusion chamber (5 ml volume) with a constant flow of physiological saline (15 ml/min flow rate). The temperature was maintained at 10 °C for cod atrium, 17 °C for carp atrium and 20 °C for atrium of frog and lizard. Thus, the test temperature always corresponded to the acclimation temperature.

In the experiments with fish, we used the isolated atrial preparation either together with the sinoatrial ring, where the pacemaker of the cod and carp heart is located (Saito, 1973; Lukyanov et al., 1986), or without it (in experiments with paced rhythm). For the species, we used preparations of right atrium without the pacemaker and stimulating electrodes were then placed near the sinoatrial junction. In all experiments with paced rhythm, the pacemaker was excised after the determination of spontaneous beating frequency. The pacing frequency was always set slightly higher than the frequency of spontaneous beats in order to avoid possible extra excitations: 1.1 Hz for the cod atrium (spontaneous frequency — 0.96 ± 0.07 Hz), 0.6 Hz for the carp atrium (0.46 ± 0.12 Hz), 1 Hz for the frog atrium (0.91 ± 0.04 Hz), and 1.6 Hz for the lizard atrium (1.44 ± 0.05 Hz).

2.1. Intracellular recordings of electric activity

The floating microelectrode technique, which was described earlier (Coraboeuf, 1969), was used with some modifications for intracellular recordings of electric activity. Transmembrane potentials were recorded with glass microelectrodes (20–30 MΩ) filled with 3 M KCl and connected to a high input impedance amplifier using 50 μm tungsten wire. This allowed the microelectrode to stay in the cell during contraction of the preparation. The signal was digitized, recorded and analyzed using specific software (L-card, Moscow, Russia; Synaptosoft, Fort Lee, NJ, USA). Spontaneously occurring APs were recorded from the endocardial surface of the preparation. ACh was applied in different concentrations (10^{-8}–10^{-5} M for frog and 10^{-7}–$5 \cdot 10^{-5}$ M for other species), 5 or 6 concentrations were tested on each preparation. The concentrations were applied in the ascending order with each concentration being applied for 6 min. A 20 min washout followed each concentration of ACh to minimize desensitization. Stable impalements were maintained during the entire period of perfusion with ACh solution. Changes in the APA and the AP duration at 50% of repolarisation (APD50) were determined.

2.2. Optical mapping

The method of optical mapping is based on the application of voltage-sensitive dyes. The molecules of such compounds embed into the cell membrane and emit fluorescence corresponding to the membrane potential during illumination with exciting light (Efimov et al., 2004). For the staining of the atrial preparations, the voltage-sensitive dye di-4-ANNEPS (D-1199) was added to the perfusing solution (10^{-6} M). After 3 min of staining, the excitation–contraction uncoupler, 2,3-butanedione monoxime (BDM, 15 mM), was added to suppress motion artifacts in optical signals caused by muscle contractions. The optical signals were recorded from a hexagonal area of the preparation 5 mm long (Fig. 3) at a rate of 1600 frames/s using a hexagonal 464-photodiode array (WuTech H-469IV, Gaithersburg, MD, USA). Optics were added to this array and consisted of the objective (focus distance — 24 mm) and the red filter of the emitted fluorescent light ($\lambda > 650$ nm). During the recording the preparation was exposed to green light (530 ± 20 nm), produced by a self-made ring array of 12 light-emitting diodes. The data were collected using a special data acquisition system (CardioPDA-III; RedShirtImaging, Decatur, GA, USA) and analyzed with a program Cardioplex v.8.2.1 (RedShirtImaging). Signals were low-pass filtered at 120 Hz. Preparation activation animations were produced and isochronal maps were plotted where the moment of dV/dt max was considered to be the moment of activation. We defined the point of preparation as inexcitable when the amplitude of optical signal decreases below 20% of control.

Measurements were started 10 min after the BDM application. The whole experiment did not exceed 1.5 h due to the reduction of the optical signal amplitude caused by the washout and/or photobleaching of the voltage-sensitive dye.

2.3. Drugs

Di-4-ANNEPS was purchased from Molecular Probes (Eugene, Oregon, USA). ACh was purchased from Sigma-Aldrich (St. Louis, MO, USA), $BaCl_2$ — from Labteh (Moscow, Russia).

2.4. Statistical analysis

Data were expressed as mean ± S.D. The effects of ACh on APD50 and APA were compared with the respective basal values of these parameters by Wilcoxon test. $p <= 0.05$ was adopted as the level of significance.

3. Results

3.1. Intracellular recordings of APs

In the experiments conducted on the cod atrium we have observed two distinct types of responses to ACh. Usually (in 11 of 15 experiments), ACh provoked a dose-dependent reduction of APA and APD50. Perfusion with 10^{-5} M and $5 \cdot 10^{-5}$ M ACh gradually decreased APA and finally led to complete suppression of AP generation (Figs. 1a,b and 2a). In 4 experiments ACh (even 10^{-5} M and $5 \cdot 10^{-5}$ M) caused just a slight reduction of APA together with marked decrease of APD50 (Figs. 1c and 2b). In one of these 4 preparations, which worked in the sinus rhythm, we observed a pause in the electric activity during the action of 10^{-5} M ACh (Fig. 1c). However, after the recovery of the pacemaker, APA was almost the same as before the cessation of electric activity. The sinus rhythm was nearly two times slower than during control. Therefore, ACh markedly affected the pacemaker, but not the working fiber. There was no qualitative difference between the effects of ACh in paced and spontaneously beating preparations.

Very similar results were obtained during experiments conducted on the carp atrium (Figs. 1d,e and 2c). Only 2 out of 7 preparations did not show ACh-dependent (10^{-5}, $5 \cdot 10^{-5}$ M) suppression of electric activity. In the other preparations ACh caused marked reduction of APD50 and APA (Figs. 1d,e and 2c). Thus, cholinergic suppression of

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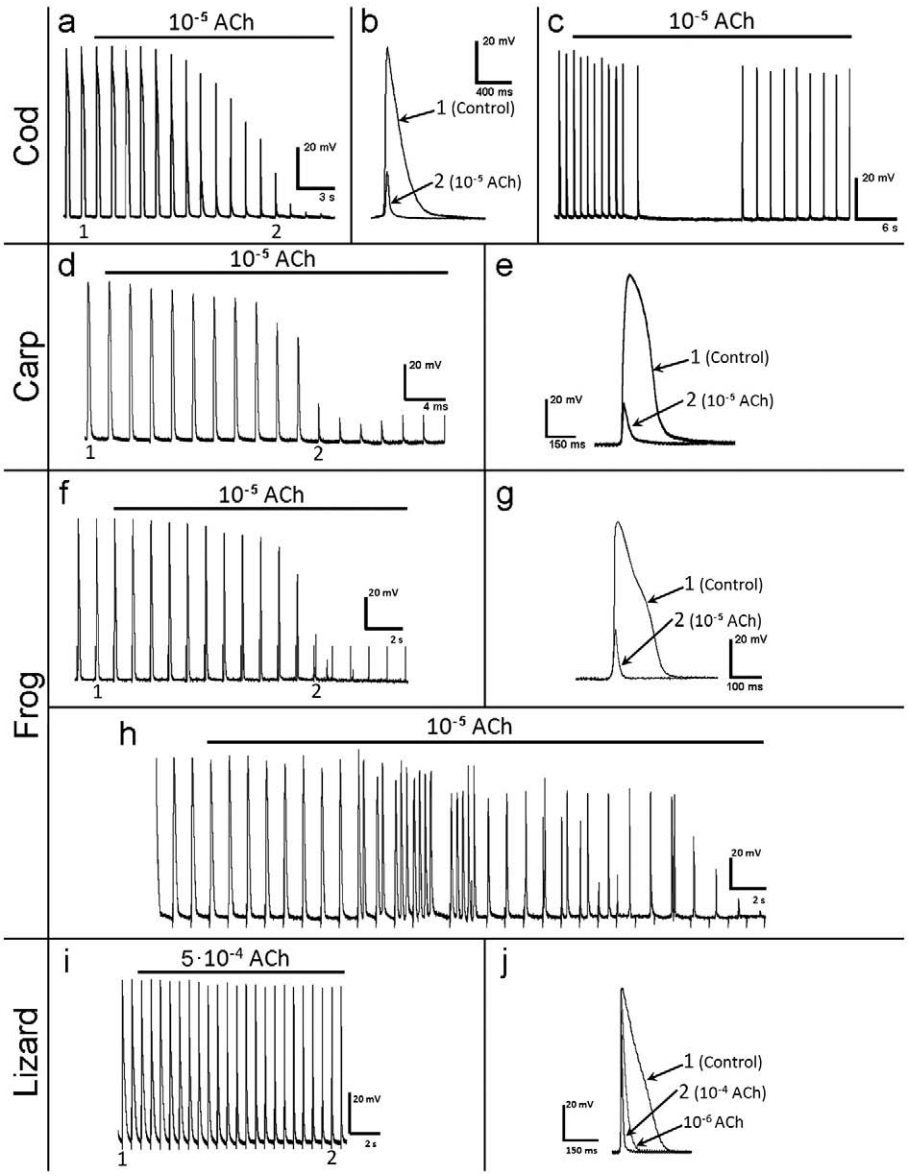

Fig. 1. Original traces of electric activity in the atrial myocardium of cod (a, b, c; sinus rhythm), carp (d, e; sinus rhythm), frog (f, g, h, pacing) and lizard (i, j, pacing). a). Suppression of AP generation under influence of 10^{-5} M ACh. b). Comparison between the APs, marked by corresponding numbers at the trace (a). c). Recovery of electric activity after a pause in the sinus rhythm, caused by 10^{-5} M ACh; APA didn't change noticeably. d). Suppression of electric activity under 10^{-5} M ACh. e). Comparison between the APs, marked by corresponding numbers at the trace (d). f). Effect of 10^{-5} M ACh. g). Comparison between the APs, marked by corresponding numbers at the trace (f). h). Development of cholinergic inexcitability during action of 10^{-5} M ACh (other experiment) coincides with episodes of atrial tachyarrhythmia. i). Changes in electric activity under $5 \cdot 10^{-4}$ M ACh. j). Comparison between the APs, marked by corresponding numbers at the trace (i) and AP, recorded during the maximal effect of 10^{-6} M ACh.

electric activity seems to be quite typical for the atrial myocardium of fishes. Analogous suppression of APA was earlier described in the frog atrium, although it was caused by vagal stimulation (Rozenshtraukh and Kholopov, 1975). Following the authors, we will term this phenomenon as cholinergic inexcitability. The question should be raised of how widespread cholinergic inexcitability is among the vertebrate animals. It is well known that ACh doesn't affect APA in mammalian atrium (Hoffman and Cranefield, 1960). Therefore, we conducted microelectrode experiments on amphibians and reptiles.

Cholinergic inexcitability similar to those described in the fish atria was observed in 8 of 9 experiments with frog right atrium. $5 \cdot 10^{-7}$ M and higher concentrations of ACh provoked significant decreases in APA, $5 \cdot 10^{-6}$ M and 10^{-5} M caused complete block of excitation (Figs. 1f,g,h and 2d). Moreover, in 2 of this 8 experiments short episodes of atrial tachyarrhythmias occurred during perfusion with ACh ($5 \cdot 10^{-6}$, 10^{-5} M) (Fig. 1 h) and subsequent washout. In one preparation 10^{-5} M ACh didn't affect APA, but decreased APD50. Thus, exogenous ACh exerted effects in the frog atrium that were similar to earlier studies using vagal stimulation.

For the experiments with reptilian atrium the most common reptilian species, lizard (*L. agilis*), was selected. ACh did not cause any detectable changes of APA in the lizard atrium, although it markedly reduced APD50 (Figs. 1i,j and 2e). For example, 10^{-5} M ACh decreased APD50 by $87.6 \pm 8.3\%$ of control value. Thus, reptilian atrial myocardium demonstrates a response to ACh similar to those in mammalian atrium. We conclude that cholinergic inexcitability of atrial myocardium is a distinctive feature of lower, anamniotic vertebrates – fishes and amphibians, but not amniotes – reptilians and mammals.

Results obtained in the experiments with fish and frog atrial myocardium indicate that some regions of atrium remain excitable during the application of high ACh concentrations. Therefore, the optical mapping technique was used to reveal the configuration and relative size of inexcitable zones in the atrium of carp and frog.

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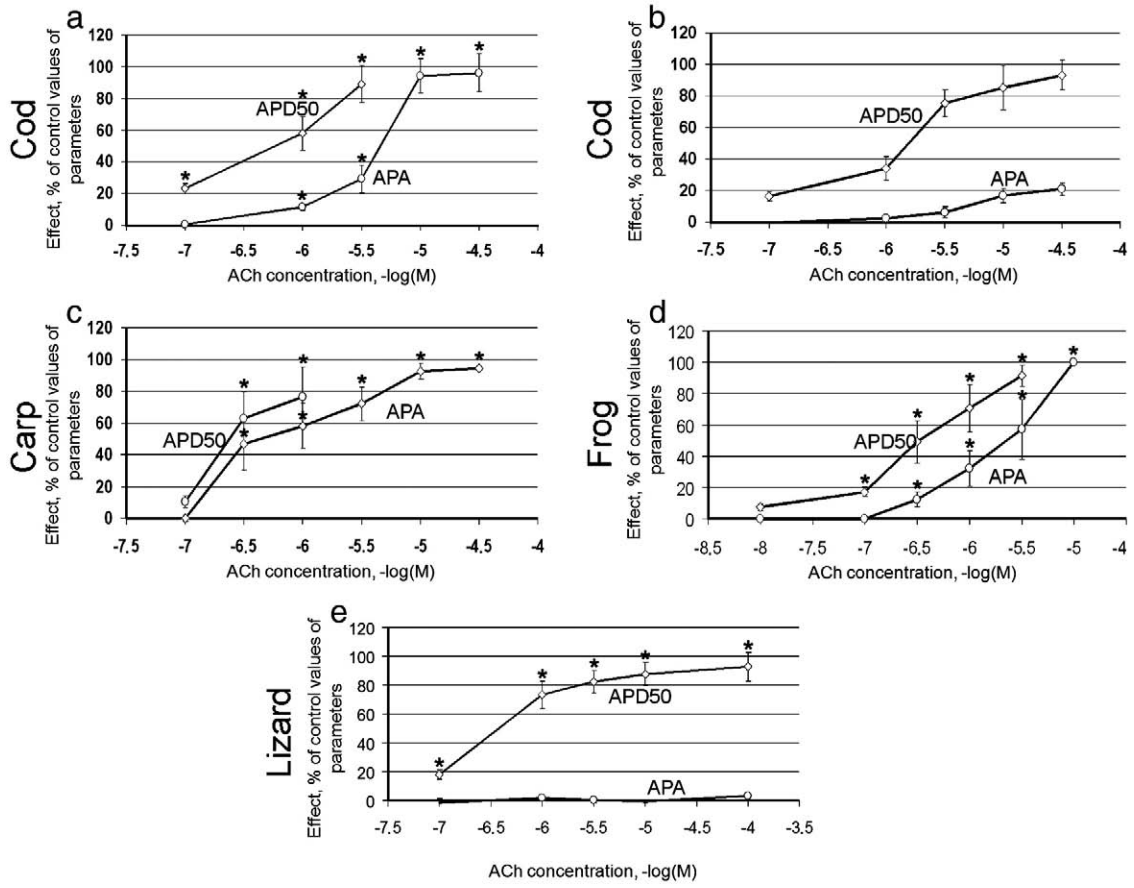

Fig. 2. Dose-dependent effects of ACh in the atrial preparations. a). Cod atrium — group of experiments with marked suppression of electric activity by ACh. Control values: APA — 97.5 ± 4.6 mV, APD50 — 216.3 ± 17.9 ($n = 11$). b). Cod atrium — group of experiments without alteration of APA by ACh. Control values: APA — 101.1 ± 3.8 mV, APD50 — 220.1 ± 12.9 ($n = 4$). c). Carp atrium. Control values: APA — 94.3 ± 5.4 mV, APD50 — 156.8 ± 22.6 ($n = 5$). d). Frog right atrium. Control values: APA — 97.0 ± 6.2 mV, APD50 — 114.6 ± 11.0 ($n = 8$). e). Lizard right atrium. Control values: APA — 95.6 ± 7.3 mV, APD50 — 103.2 ± 9.5 ($n = 7$). Ordinates: % decrease in APD50 and APA. *$p < 0.05$ vs. the respective control values.

3.2. Optical mapping

The size of carp atrium preparation is approximately 13 × 13 mm, therefore we could conduct recording of optical signals only in a part of the preparation. The area nearest to the sinoatrial valve was chosen for the registration.

A representative example of carp atrial myocardium activation sequence during normal conditions is shown at Fig. 3b. ACh (10^{-5} M) caused a gradual decrease of the optical signal amplitude (Fig. 3a) down to the full cessation of the signal in the majority of the mapped part of atrium (62 ± 18% of the entire square of the mapped area, $n = 6$) (Fig. 3c). In the rest of the mapped area optical signal persisted.

In each experiment we have also tested ACh at the concentration of $5 \cdot 10^{-4}$ M. In these hearts, preparations were paced with a frequency of 1 Hz, because such a high concentration of ACh ceases the activity of the pacemaker. In all 6 preparations the region proximal to the sinoatrial valve (31 ± 12% of the entire square, $n = 6$) remained excitable. Thus, we suppose, that the carp atrium contains two types of myocardium distinguished by response to high concentrations of ACh.

In the experiments with a frog right atrium we have mapped about 60% of its whole square. Like in the carp atrium, 10^{-5} M ACh caused formation of vast inexcitable region (57.5 ± 9% of the entire square, $n = 6$) (Fig. 4). Perfusion with $5 \cdot 10^{-4}$ M ACh led to the onset of larger inexcitable zone (72.5 ± 5% of the entire square, $n = 6$), although the other myocardium, which bordered upon the sinoatrial junction, remained active. Thus, two types of response to ACh are present in the frog atrial myocardium as well as in carp.

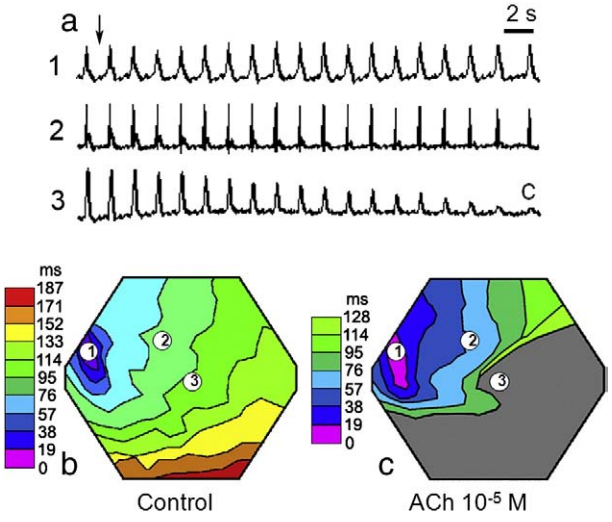

Fig. 3. Optical mapping of the carp atrium fragment (single representative experiment): changes of the activation sequence under 10^{-5} M ACh. a). Traces of optical signals that were registered in the points marked by numbers on maps (b) and (c). Arrow indicates the beginning of 10^{-5} M ACh application. b). Isochronal map of the carp atrium fragment: activation under control conditions. Magenta color indicates the region of the earliest excitation (here and in all other figures). c). Map of activation of the same carp atrium fragment during the cycle marked by the letter "c" at (a). The region with suppressed electric activity (optical signal amplitude is less than 40% of control amplitude) is grey (here and in all subsequent figures). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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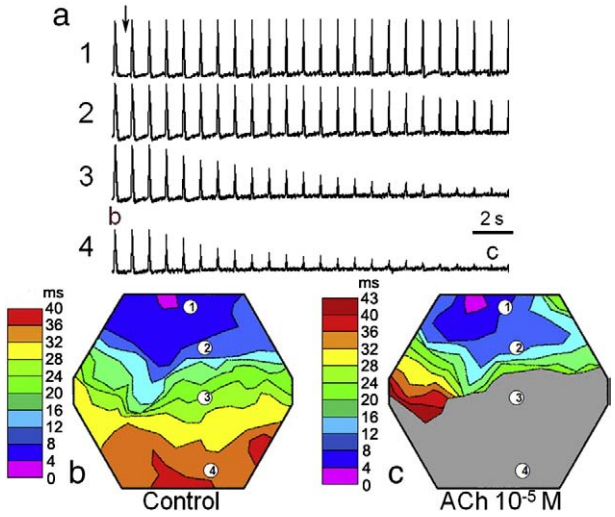

Fig. 4. Optical mapping of the frog right atrium fragment (single representative experiment): changes of the activation sequence under 10^{-5} M ACh. a). Traces of optical signals that were registered in the points marked by numbers on maps (b) and (c). Arrow indicates the beginning of 10^{-5} M ACh application. b). Isochronal map of the frog atrium fragment: activation under control conditions during the cycle marked by letter "b" at (a). c). Map of activation of the same carp atrium fragment during the cycle marked by letter "c" at (a).

Short episodes of atrial fibrillation were observed under the action of 10^{-5} M ACh in 2 preparations during the experiments with optical mapping of the frog atrium.

To reveal the possible ionic mechanisms of cholinergic inexcitability, we have performed experiments with barium chloride. Ba^{2+} blocks acetylcholine-dependent potassium channels (I_{KACh}) and background inward rectifier potassium channels (I_{K1}) (Boyett et al., 1995). Authors used barium chloride to investigate the contribution of I_{KACh} to the effects of ACh in the rabbit sinoatrial node. In a special series of experiments with optical mapping of the frog right atrium we have applied $BaCl_2$ (10^{-4} M) 5 min before and during perfusion with 10^{-5} M ACh. In the control conditions ACh caused suppression of electric activity in a part of the atrium (Fig. 5a,c). After a 15-minute washout, $BaCl_2$ was applied. $BaCl_2$ caused changes in the configuration of the optical signal: reduction of amplitude and prolongation of APs, although it is not possible to analyze these changes in terms of quantity. $BaCl_2$ completely prevented onset of cholinergic inexcitability (Fig. 5b,d) in all 6 experiments. We did not observe changes in the optical signal amplitude during the application of 10^{-5} M ACh (Fig. 5b) and the activation sequence also remained unchanged.

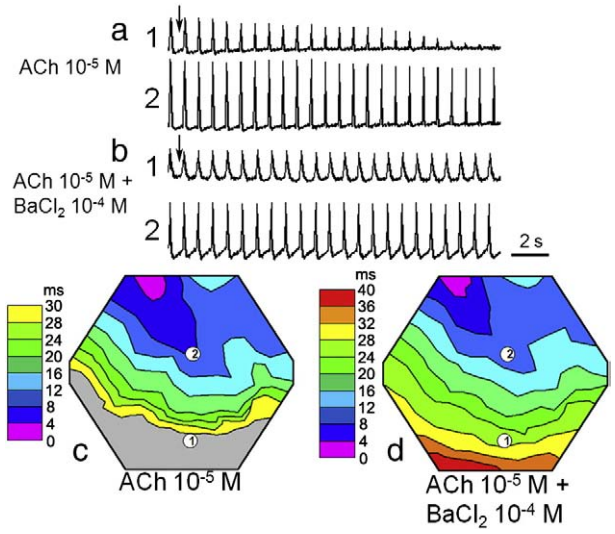

Fig. 5. Optical mapping of the frog right atrium fragment (single representative experiment different to presented at Fig. 4): $BaCl_2$ prevents suppression of electric activity by 10^{-5} M ACh. a). Traces of optical signals that were registered during action of 10^{-5} M ACh in the points marked by numbers on maps (c) and (d). Arrow indicates the beginning of 10^{-5} M ACh application. b). Traces of optical signals that were registered in the same points during action of 10^{-5} M ACh in the presence of 10^{-4} M $BaCl_2$. c). Activation during the last cycle shown at (a). d). Activation of the same fragment during the last cycle shown at (b).

4. Discussion

In the present study we demonstrate the phenomena of cholinergic inexcitability and suppression of electric activity by high concentrations of ACh in the atrial myocardium of cod, carp and frog. This is the first detailed study of cholinergic inexcitability in the fish, amphibian and reptilian atrium conducted using the mapping technique. Reduction of APA by ACh was reported in the study of Molina et al. (2007), but this observation was not discussed further. In the frog atrium, cholinergic suppression of APA was demonstrated in our earlier study (Rozenshtraukh and Kholopov, 1975) and ionic mechanisms of this phenomenon were investigated by Giles and Noble (1976).

Cholinergic inexcitability seems to be deserving of attention as a special feature of lower vertebrate animal hearts. ACh never alters APA in the atrial myocardium of mammals (Hoffman and Cranefield, 1960) or, as we have demonstrated, reptiles. It may be proposed that cholinergic inexcitability of working atrial myocardium is a specific characteristic of more evolutionary primitive vertebrates — fishes and amphibians, while in the higher forms this phenomenon disappeared during subsequent evolution. We may speculate that the major part of fish and amphibian atrial myocardium does not have enough sodium and Ca^{2+} channels to preserve excitability during the vigorous activation of outward ACh-dependent potassium current. The density of inward current channels may be higher in the pacemaker and special conductive trabecules, which connect the sinus venosus (or sinoatrial ring in fish) and the atrioventricular junction. Being more advanced, the atrial myocardium of reptiles and mammals might possess larger density of sodium and Ca^{2+} channels and therefore lack cholinergic inexcitability. This seems to provide more effective atrial function in higher vertebrate animals. However, this is only a speculation and needs to be proven in appropriate experiments.

Together with the results of microelectrode experiments, the optical mapping data indicate that while the majority of atrial myocardium of carp and frog becomes inexcitable under the action of ACh, a small region located close to the sinoatrial junction preserves excitability. It may be hypothesized that the fibers which remain excitable may be necessary for faster conduction of excitation from the sinoatrial border to the atrioventricular junction. When ACh is applied, they may continue to conduct the excitation, while other parts of the atrial myocardium may be "switched off".

It is well known that ACh alters the AP configuration in the myocardium by modulation of different ionic currents via stimulation of muscarinic receptors. It activates outward I_{KACh}, but suppresses the L-type Ca^{2+} current (I_{CaL}) and funny current (I_f) (Harvey and Belevych, 2003). Prevention of cholinergic inexcitability by barium chloride, a potent blocker of I_{KACh} suggests a crucial role of I_{KACh} in this phenomena. Although, barium chloride is not a selective I_{KACh} blocker and also affects I_{K1}, this latter current doesn't contribute to the ACh action. ACh is not shown to affect I_{K1} (Harvey and Belevych, 2003), so the suppression of APA by ACh can't be related to I_{K1} alteration. Therefore, we suppose that the activation of I_{KACh}, which reduces the effect of inward currents on membrane potential, is one of the mechanisms of cholinergic inexcitability, in at least the frog atrial myocardium.

There are several limitations of our study. Firstly, the experimental temperature was the same as the acclimation temperature to avoid

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the possible artifacts caused by non-physiological temperature. However, temperature may alter the proarrhythmic ability of ACh greatly (Lin et al., 2000). Therefore, our protocol limits the direct comparison between different species. Second, the frequency of spontaneous excitations and pacing frequency also differed among the species. Third, experiments with more selective blocker of I_{KACh} are required to confirm our conclusion about crucial role of I_{KACh} in the genesis of cholinergic inexcitability.

The physiological role of cholinergic inexcitability is not clearly understood. However, its possible role in the mechanism of cholinergic arrhythmia initiation has been proposed (Rosenshtraukh et al., 1989; Rosenshtraukh and Zaitsev, 1990). According to this hypothesis, inexcitable zones serve as a substrate for the formation of re-entry circuits, because the wave of excitation may go around one of the inexcitable regions (Rosenshtraukh and Zaitsev, 1990). Although we didn't observe atrial arrhythmia in the experiments on fish atrium, we did record several episodes of tachyarrhythmia during the experiments on frog atrium. Therefore the hypothesis concerning correlation of cholinergic inexcitability and atrial arrhythmias is likely to be confirmed, at least in the case of frog. This finding seems to be of interest, because cholinergic inexcitability has been earlier described in the mammalian sinoatrial node (Vinogradova et al., 1998; Fedorov et al., 2006). Further investigations should reveal the possible arrhythmogenic significance of cholinergic inexcitability in the mammalian heart.

This study demonstrates the presence of the cholinergic APA suppression in the atrial myocardium of fishes and amphibians in contrast to mammals and reptilians. We hope that this report will encourage further research of physiological role of this phenomenon, its contribution to arrhythmogenesis and its ionic mechanisms.

Acknowledgements

Authors are grateful to Prof. Tsetlin A.B. for the invaluable support of this study. The project was supported by President of Russia Grant (Scientific Schools—4417.2008.7).

References

Allessie, M.A., Bonke, F.I., Schopman, F.J., 1977. Circus movement in rabbit atrial muscle as a mechanism of tachycardia. 3. The “leading circle” concept: a new model of circus movement in cardiac tissue without the involvement of anatomical obstacle. Circ. Res. 41, 9–18.

Boyett, M.R., Kodama, I., Honjo, H., Arai, A., Suzuki, R., Toyama, J., 1995. Ionic basis of the chronotropic effect of acetylcholine on the rabbit sinoatrial node. Cardiovasc. Res. 29, 867–878.

Coraboeuf, E., 1969. Resistance measurements by means of microelectrodes in cardiac tissues. In: Lavallee, M., Schanne, O.F., Habert, N.C. (Eds.), Glass Microelectrodes. Wiley, New York, pp. 224–271.

Coyne, M.D., Kim, C.S., Cameron, J.S., Gwathmey, J.K., 2000. Effects of temperature and calcium availability on ventricular myocardium from rainbow trout. Am. J. Physiol. Regul. Integr. Comp. Physiol. 278, 1535–1544.

Efimov, I.R., Nikolski, V.P., Salama, G., 2004. Optical imaging of the heart. Circ. Res. 95, 21–33.

Fedorov, V.V., Hucker, W.J., Dobrzynski, H., Rosenshtraukh, L.V., Efimov, I.R., 2006. Postganglionic nerve stimulation induces temporal inhibition of excitability in rabbit sinoatrial node. Am. J. Physiol, Heart Circ. Physiol. 291, 612–623.

Franklin, C.E., Axelsson, M., 1994. The intrinsic properties of an in situ perfused crocodile heart. J. Exp. Biol. 186, 269–288.

Giles, W., Noble, S.J., 1976. Changes in membrane currents in bullfrog atrium produced by acetylcholine. J. Physiol. 261, 103–123.

Harvey, R.D., Belevych, A.E., 2003. Muscarinic regulation of cardiac ion channels. Brit. J. Pharmacol. 139, 1074–1084.

Hoffman, B.F., Cranefield, P., 1960. Electrophysiology of the Heart. McGraw-Hill Book Co, New York.

Hoglund, L., Gesser, H., 1987. Electrical and mechanical activity in heart tissue of flounder and rainbow trout during acidosis. Comp. Biochem. Physiol. 87A (3), 543–546.

Lin, T., Hou, Z., Liu, H., Wu, H., Lin, C., 2000. Atrial tachyarrhythmias induced by acetylcholine in tilapia (*Oreochromis* sp.) isolated atria. Clin. Exp. Pharmacol. Physiol. 27, 330–338.

Lukyanov, A.N., Sukhova, G.S., Chudakov, L.I., 1986. Mechanism of variation in the general rhythm of the heart pacemaker in the cod *Gadus morhua* in response to vagal stimulation. J. Evol. Biochem. Physiol. 22 (1), 25–30.

Molina, C.E., Gesser, H., Llach, A., Tort, L., Hove-Madsen, L., 2007. Modulation of membrane potential by an acetylcholine-activated potassium current in trout atrial myocytes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292, 388–395.

Nadeau, R.A., Roberge, F.A., Billette, J., 1970. Role of the sinus node in the mechanism of cholinergic atrial fibrillation. Circ. Res. 27, 129–138.

Rosenshtraukh, L.V., Zaitsev, A.V., 1990. Atrial tachycardias — a new look. News Physiol. Sci. 5, 187–190.

Rosenshtraukh, L.V., Zaitsev, A.V., Fast, V.G., Pertsov, A.M., Krinsky, V.I., 1989. Vagally induced block and delayed conduction as a mechanism for circus movement tachycardia in frog atria. Circ. Res. 64 (2), 213–226.

Rozenshtraukh, L.V., Kholopov, A.V., 1975. Role of the vagus nerves in the origin and cessation of atrial tachyarrhythmias. Kardiologiya 15 (1), 38–48.

Saito, T., 1973. Effects of vagal stimulation on the pacemaker action potentials of carp heart. Comp. Biochem. Physiol. 44A, 191–199.

Vinogradova, T.M., Fedorov, V.V., Yuzyuk, T.N., Zaitsev, A.V., Rosenshtraukh, L.V., 1998. Local cholinergic suppression of pacemaker activity in the rabbit sinoatrial node. J. Cardiovasc. Pharmacol. 32, 413–424.

Please cite this article as: Abramochkin, D.V., et al., Cholinergic modulation of activation sequence in the atrial myocardium of non-mammalian vertebrates, Comp. Biochem. Physiol. A (2009), doi:10.1016/j.cbpa.2009.11.002