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ORIGINAL PAPER

# Bioelectrical activity in the heart of the lugworm *Arenicola marina*

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**Abstract** Standard microelectrode technique was used to study electrical activity of the isolated heart of the polychaete annelid, *Arenicola marina*. Typical pacemaker activity with slow diastolic depolarization was observed in all recordings. The average maximum diastolic potential ($-58.4 \pm 3.2$ mV), the average amplitude of the action potential ($28.7 \pm 4.7$ mV) and the average total duration of the action potential ($2{,}434 \pm 430$ ms) were determined. There has been no gradient of automaticity observed in our studies, which suggests that all regions of the *Arenicola* heart could possess pacemaker functions. Acetylcholine (ACh) produced a concentration dependent ($5 \times 10^{-8}$–$5 \times 10^{-5}$ M) increase of the beating rate via increase in the rate of the diastolic depolarization. ACh ($5 \times 10^{-5}$ M) increased beating rate by 2.5-fold compared to the control rate. A stronger action of ACh resulted in depolarization, block of action potential generation and contracture of the heart. The non-hydrolysable ACh analog carbacholine ($10^{-8}$–$10^{-6}$ M) produced similar effects. All effects of ACh and carbacholine were abolished by $5 \times 10^{-6}$ M atropine. D-Tubocurarine ($5 \times 10^{-5}$ M) did not significantly alter effects of ACh or carbacholine. Epinephrine ($10^{-8}$–$10^{-6}$ M) caused the slowing of pacemaker activity and marked decrease of action potential duration. $10^{-6}$ M epinephrine produced complete cardiac arrest. The effects of epinephrine were not significantly altered by the $\beta$-blocker propranolol ($5 \times 10^{-6}$ M). The $\beta$-agonist isoproterenol ($10^{-7}$–$10^{-5}$ M) and the $\alpha$-agonist xylometazoline ($10^{-6}$–$10^{-5}$ M) did not produce significant effects. Thus, cholinergic effects in the *Arenicola* heart are likely to be mediated via muscarinic receptors, while the nature of adrenergic effects needs further investigation.

**Keywords** Annelida · Lugworm · *Arenicola* · Heart · Action potential · Acetylcholine

**Abbreviations**

| | |
|---|---|
| ACh | Acetylcholine |
| AP | Action potential |
| APD50 | Action potential duration to 50% of repolarization |

## Introduction

During the course of the evolution of invertebrate animals, Annelida was the first phylum that has a fully developed heart as a specialized organ that pumps blood with stable rhythmic activity. Although, polychaete annelids have well-developed closed circulatory system with ventral and dorsal longitudinal vessels (Prosser et al. 1951) that can perform pumping function in most of the species, only few species among polychaete and oligochaete worms have developed specialized hearts to improve their blood pumping capabilities. In Hirudinea annelids two lateral heart tubes are present, which run longitudinally along the entire length of an animal (Maranto and Calabrese 1984a).

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The two hearts of *Arenicola marina* are oval muscular organs attached to the dorsolateral walls of the stomach, one on each side (Fig. 1). Blood enters the heart from the gut sinus and then flows to the ventral longitudinal vessel. This heart has only one chamber, which consists of two layers of myoepithelial cells and a layer of extracellular matrix between them (Martynova and Chaga 2002). These cells are 5–10 μm in diameter, non-striated and contain thick and thin filaments and Z-bodies (Jensen 1974).

The physiology of the *Arenicola* heart is poorly investigated. One of the hypotheses suggests a neurogenic nature of the electrical activity of *Arenicola* heart. It was proposed because of the stimulatory effect of acetylcholine (ACh) and some specific extracellular recordings of the electrical activity of the heart (Prosser and Zimmerman 1943; Prosser 1950; Prosser et al. 1951). Although modern morphological studies haven't described innervation in this heart (Martynova and Chaga 2002), it is still unclear whether its electrical activity has myogenic or neurogenic nature. In our earlier study, we have also demonstrated the ability of the *Arenicola* heart to generate a spontaneous rhythm which was independent of its filling with blood (Tennova et al. 1982).

Among annelids, only leech heart physiology has been extensively investigated in several studies. Leech hearts are myogenic and isolated myocytes are capable of generating their own spontaneous rhythm, but the heartbeat pattern is driven by a central pattern generator (Maranto and Calabrese 1984b, c).

In this study, we show intracellular recordings of the electrical activity of *Arenicola* heart that are similar to that of some invertebrates with myogenic hearts, especially the heart of mussel, as well as to that of vertebrate pacemaker cardiomyocytes. We have also studied the stimulating effect of acetylcholine (ACh) and the inhibitory effect of epinephrine on action potentials (APs) generation in *Arenicola* heart. Surprisingly, the pattern of electrical activity in *Arenicola* heart and the receptor mechanisms of cholinergic effects were found to be quite different from those shown in other annelids with developed hearts, such as leeches.

## Materials and methods

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

Experiments were performed at the White Sea Biological Station of Moscow State University (Karelia, Russia). Adult polychaete annelids *Arenicola marina* L. were collected at the littoral near the station in July–September. The animals were kept in an aquarium with running sea water (15°C) before experiment.

### Registration of electrical activity

Worms were fixed in the preparation dish and a longitudinal section of the muscular wall was made (Fig. 1). After 30 min, both hearts were separated from the stomach and the efferent vessel, which connects the heart with the ventral longitudinal vessel, was ligatured. After the heart was filled with blood, the afferent vessel was also ligatured. Finally the heart was removed and placed in the experimental chamber with 3 ml of artificial sea water containing NaCl 335.7 mM, KCl 7.25 mM, $MgSO_4$ 17.7 mM, $MgCl_2$ 21.4 mM and $CaCl_2$ 6.58 mM, and bubbled with $O_2$. pH was adjusted to 7.6 ± 0.2 with 1 N NaOH and 1 N HCl. The heart was superfused at 15°C with a flow rate of 12 ml/min. Work of the isolated heart remained stable for 3–4 h with rhythm fluctuations that did not exceed 15% of the rate after isolation. Both hearts were isolated from the animal but only the most stable of the two hearts was used in the final experiment.

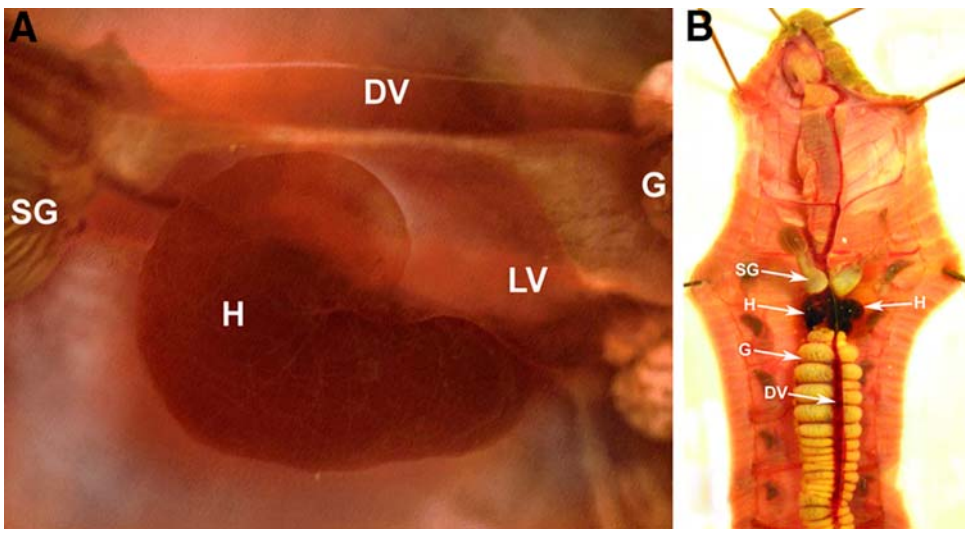


**Fig. 1** The location of hearts in the dissected lugworm *Arenicola marina*. **a** Photograph of the right heart. **b** General view of the dissected worm. *H* heart, *DV* dorsal vessel, *LV* vessel of lateral (gastral) sinus, *G* gut, *SG* salivary gland

The floating microelectrode technique that has been described earlier (Coraboeuf 1969) was used with some modifications for the intracellular electrical activity registration. Transmembrane potentials were recorded with glass microelectrodes (20–30 MΩ) filled with 3 M KCl and connected to the high input impedance amplifier using 50 μm tungsten wire. This configuration allowed microelectrode to stay inside a cell during contraction of the heart. The signal was digitized, recorded and analyzed using specific software (L-card, Moscow, Russia; Synaptosoft, Fort Lee, NJ, USA). Spontaneous APs were registered from the outer heart surface. Stable impalements were maintained during the entire period of drug action. However, it was quite difficult to obtain such stable impalement due to the high amplitude of the heart contractions and the small size of the cells. The rate of successful experiments was 64.4%. Changes in the beating rate of the heart and the AP duration at 50% of repolarization (APD50) were determined.

### Drugs

ACh chloride, carbacholine chloride, atropine, D-tubocurarine chloride hydrate, epinephrine, propranolol hydrochloride, xylometazoline hydrochloride and isoproterenol were purchased from Sigma (St. Louis, MO, USA).

### Statistical analysis

A total of 87 animals were used in this study, but only 56 experiments were successful. Data are expressed as mean ± S.D. The effects of ACh, carbacholine, epinephrine and other agonists on APD50 and heart rate were compared with respective basal values of APD50 and heart rate using the Wilcoxon test. The effects of ACh and other agonists alone and in the presence of different antagonists were compared using a Mann–Whitney test. $P \leq 0.05$ was adopted as the level of significance.

## Results

### Morphology of the electrical activity of the *Arenicola* heart

The pattern of electrical activity varied greatly among the cells of the *Arenicola* heart. Different types of activity are shown in Fig. 2. However, the membrane potential was not stable in all cells during diastole, and slow diastolic depolarization was always observed. Maximal diastolic potential was −58.4 ± 3.2 mV ($n = 22$).

AP amplitude also varied among the cells and the hearts; the average amplitude of AP was 28.7 ± 4.7 mV ($n = 22$).

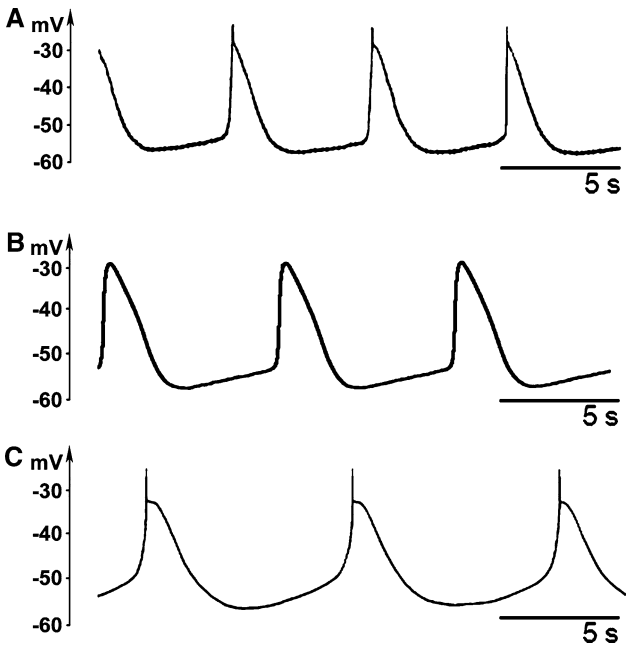


**Fig. 2** Original traces of APs recorded from different hearts. **a**, **b** APs in latent pacemaker cells. **c** APs in primary pacemaker cells

Positive values of the peak potential were never registered. AP duration varied from 1,200 to 4,000 ms, with an average duration of 2,434 ± 430 ms. Average AP upstroke velocity was 0.36 ± 0.04 V/s. Input resistance of the cells was about 12–14 MΩ. This relatively low input resistance could possibly be explained by the electrical coupling between myocytes within the heart (Maranto and Calabrese 1984a) but further investigation is needed to obtain accurate data for the cell coupling inside the heart tissue.

Some cells showed a smooth transition between diastolic depolarization and the AP upstroke (Fig. 2c) but in some cells, such transition was quite abrupt. However, we could not determine any specific morphological location for these two different types of electrical activity; both of these types of activity were observed throughout the heart. Moreover, in six experiments, we found that the change of activity from one type to another occurred during the long AP registration. In seven additional experiments, we cut the heart into two pieces and studied each part separately. We found that each piece was able to generate its own rhythm. The beating rate of the upper part was higher in four out of seven hearts, and in the other three hearts the beating rate was higher in the lower part. Thus, there seems to be no gradient of automaticity in the *Arenicola* heart.

### Cholinergic effects

ACh was tested in concentrations from $5 \times 10^{-8}$ M up to $5 \times 10^{-5}$ M followed by 30-min washouts after each ACh application. ACh caused dose-dependent increase in

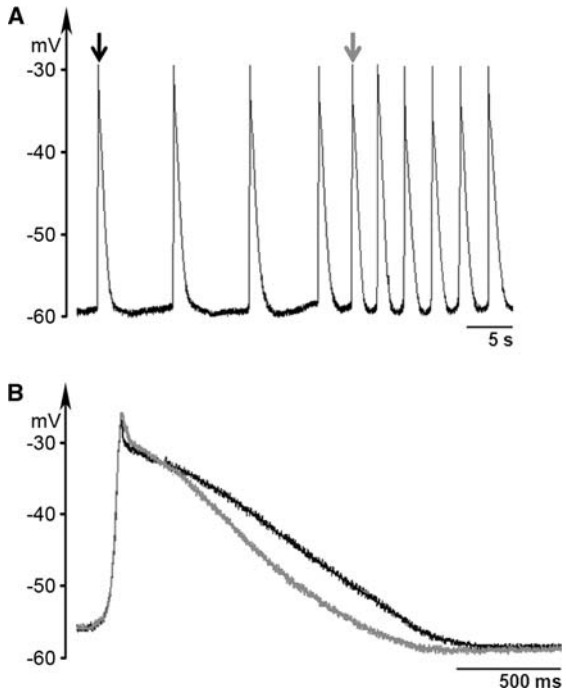


**Fig. 3** Original traces of APs during the action of $10^{-5}$ M ACh. **a** Electrical activity during the action of $10^{-5}$ M ACh, the *first arrow* marks the moment when solution of ACh came to the experimental chamber. **b** Changes in AP configuration induced by ACh: APs which are marked in **a** by *arrows* are superimposed

the beating rate of the *Arenicola* heart (Figs. 3, 4a). A representative example of $10^{-5}$ M ACh effect is shown in Fig. 3a. $5 \times 10^{-5}$ M ACh caused a prolongation of the AP depolarization and arrest of heart activity in systole in five out of six experiments. The average maximum increase of beating rate during the action of $5 \times 10^{-5}$ M ACh was 254.5 ± 37.7%, compared to the control heart rate. Other concentrations did not significantly affect the AP amplitude. Only the high ACh concentrations ($5 \times 10^{-6}$ M–$5 \times 10^{-5}$ M) caused a significant reduction in AP duration (Figs. 3b, 4a): $5 \times 10^{-6}$ M reduced APD50 by 12.2 ± 1.9% of the control APD50; $5 \times 10^{-5}$ M by 14.7 ± 1.7% ($n = 6$). During the ACh effect, diastolic depolarization rate was increased and in some cases, ACh caused a transformation of the transition between diastolic depolarization and the AP upstroke from abrupt to smooth.

All effects of ACh were mimicked by its non-hydrolysable analog carbacholine ($10^{-8}$–$10^{-6}$ M) (Fig. 4b, c). $10^{-6}$ M carbacholine stopped the heart right after excessive rhythm increase. In additional series of experiments, the muscarinic antagonist atropine ($5 \times 10^{-6}$ M) was applied 5 min before adding $10^{-5}$ M ACh or $10^{-6}$ M carbacholine. Atropine completely abolished all effects of ACh and carbacholine (Fig. 4c). In another set of experiments the nicotinic antagonist D-tubocurarine ($5 \times 10^{-5}$ M) did not significantly alter the effects of ACh and carbacholine (Fig. 4c).

Thus, the cholinergic agonists markedly increase the beating rate of the heart and slightly reduce APD of the isolated heart of *Arenicola marina*. All effects of ACh and carbacholine were blocked by atropine, but not by D-tubocurarine.

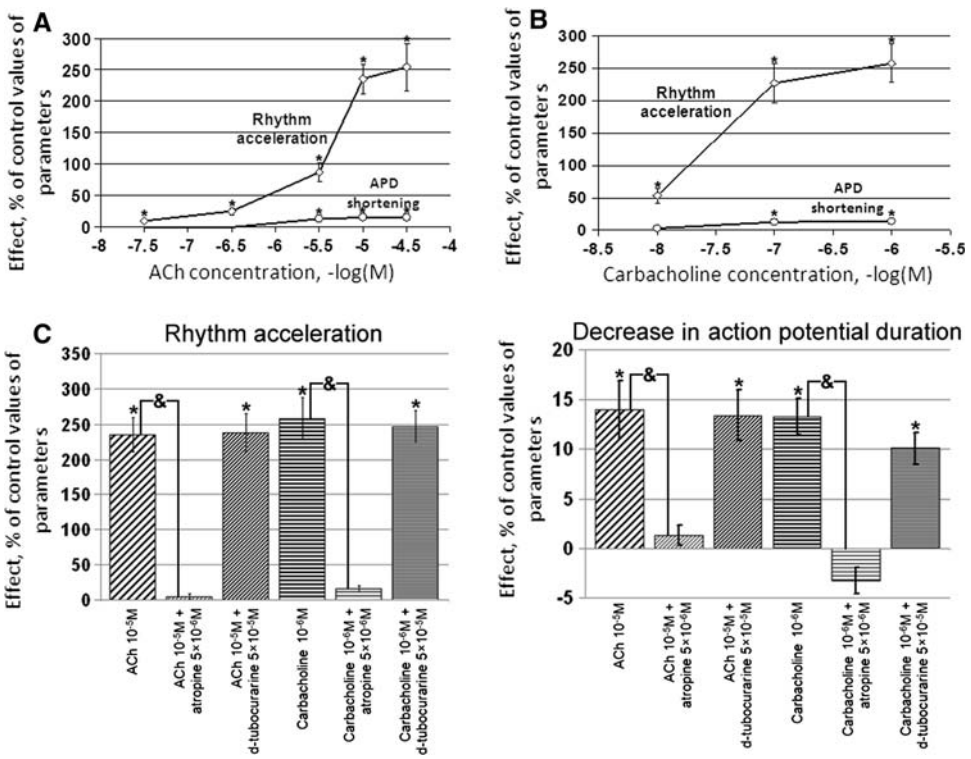


**Fig. 4** Cholinergic effects on the isolated *Arenicola* heart. Ordinates: % increase in beating rate or % decrease in APD. **a** Dose-dependent effects of ACh ($n = 6$). **B** Dose-dependent effects of carbacholine ($n = 6$). **c** Influence of muscarinic antagonist atropine ($n = 6$) and nicotinic antagonist D-tubocurarine ($n = 6$) on the effects of ACh and carbacholine. *$P < 0.05$ versus the respective control values, Wilcoxon test. & $P < 0.05$ effects of ACh or carbacholine in the presence of atropine versus ACh or carbacholine alone, Mann–Whitney test

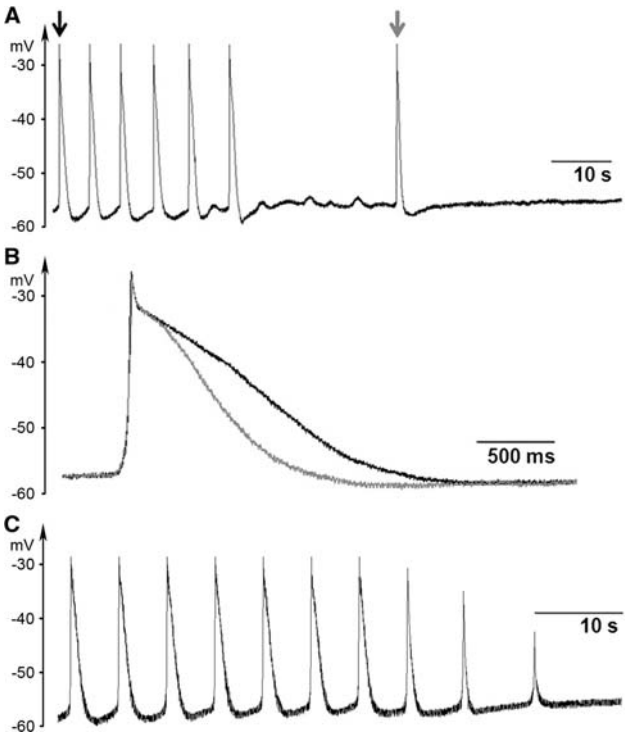


**Fig. 5** Original traces showing effect of epinephrine in high concentrations. **a** electrical activity during the action of $5 \times 10^{-7}$ M epinephrine, the *first arrow* marks the moment when solution of epinephrine reached the experimental chamber. **b** Changes in the AP shape induced by epinephrine: APs which are marked by *arrows* in **a** are superimposed. **c** Electrical activity during the action of $10^{-6}$ M epinephrine; epinephrine causes a reduction of AP amplitude

### Adrenergic effects

Epinephrine was studied in concentrations from $10^{-8}$ M up to $10^{-6}$ M, followed by 30-min washouts after each epinephrine application. Epinephrine caused a dose-dependent reduction in the beating rate of the *Arenicola* heart (Figs. 5, 6a). A representative example of a $5 \times 10^{-7}$ M epinephrine effect is shown in Fig. 5a. In four out of six experiments, $5 \times 10^{-7}$ M epinephrine caused a cardiac arrest during the diastole phase of the heart cycle. At $10^{-6}$ M, epinephrine stopped the heart in all experiments. Slowing of rhythm was accompanied by the reduction of diastolic depolarization rate. We have also found a marked reduction of APD during application of epinephrine (Figs. 5b, 6a). In four experiments, $5 \times 10^{-7}$ M and $10^{-6}$ M epinephrine also caused a gradual suppression of AP amplitude before the final cessation of the heart activity (Fig. 5c). However, lower epinephrine concentrations, which did not suppress the electrical activity completely, had no effect on the AP amplitude.

A synthetic agonist of $\beta$-adrenoreceptors, isoproterenol ($10^{-7}$ and $10^{-6}$ M) did not significantly alter the heart rhythm and APD (Fig. 6b). Higher concentration of isoproterenol ($10^{-5}$ M) caused minor increase of the rhythm. Agonist of $\alpha$-adrenoreceptors, xylometazoline ($10^{-6}$ and $10^{-5}$ M) had no significant effect on the heart function (Fig. 6c). In another series of experiments, $\beta$-antagonist propranolol ($5 \times 10^{-6}$ M) was used 5 min before adding epinephrine ($10^{-7}$ M). Propranolol did not alter the effects of epinephrine at all (Fig. 6c).

Thus, epinephrine reduces the heart rate and markedly reduces APD in the isolated heart of *Arenicola marina*. However, selective synthetic adrenomimetics did not reproduce these effects.

## Discussion

In this study, we demonstrate intracellular recording of the electrical activity of the heart of a polychaete annelid. The structure of *Arenicola* heart's electrical activity closely resembles electrical activity in the hearts of bivalve mollusks (Shigeto 1970), insects (Hertel and Pass 2002), some primitive crustaceans (Yamagishi 2003) and even, in part, mammalian sinoatrial node (West 1955). The presence of the diastolic depolarization, maximal diastolic potential near −50–60 mV, rather small amplitude of AP (about 25–30 mV) and absence of overshoot is typical for the pacemaker cells of all these myogenic hearts. So, the electrical activity of *Arenicola* heart markedly differs from the myogenic activity of the heart cells from other annelids, such as leeches, that have rhythmic bursts consisting of a slow potential with several superimposed spikes (Maranto and Calabrese 1984b). No typical excitatory junctional potentials, which are the specific attribute of neurogenic hearts (Sakurai and Yamagishi 2000), were observed in *Arenicola* heart. Rapid potential rising in the beginning of AP is likely to be rather a spike of AP than a junctional potential, note that it appears at the end of the slow rising of AP. Some cells that we observed in our experiments were without this rapid rising of potential (Fig. 2b). Moreover, according to some morphological studies, no nerve elements were found in these hearts. The ability of isolated *Arenicola* heart to work stable for several hours could also be indicative of its myogenic rhythm nature. Thus, we suggest that the automaticity of *Arenicola* heart has a myogenic nature.

All cells in the *Arenicola* heart demonstrate the main attribute of pacemaker activity which is confirmed by the presence of the diastolic depolarization. It is generally accepted that in the mammalian sinoatrial node, smooth transition is typical for the primary pacemaker site cells and sharp transition is registered in other parts of the node that are driven by the leading pacemakers (Boyett et al. 2000). So, we propose that the cells of *Arenicola* heart with a smooth transition also could represent the primary

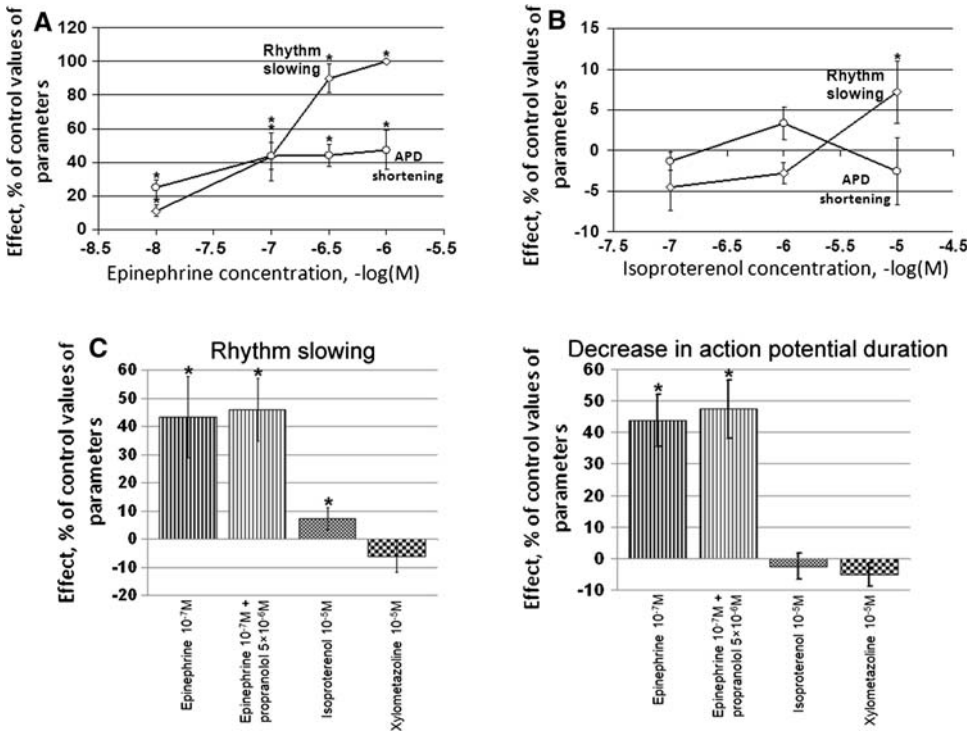


**Fig. 6** Adrenergic effects on the isolated *Arenicola* heart. Ordinates: % decrease in beating rate or APD. **a** Dose-dependent effects of epinephrine ($n = 7$). **b** Dose-dependent effects of isoproterenol ($n = 6$). **c** Effects of $\beta$-adrenoblocker propranolol ($n = 7$); agonist of $\beta$-adrenoreceptors, isoproterenol ($n = 6$); and agonist of $\alpha$-adrenoreceptors, xylometazoline ($n = 6$). $^*P < 0.05$ versus the respective control values, Wilcoxon test

pacemaker cell of the *Arenicola* heart. Moreover, we believe that most of the cells may act as a primary pacemaker and location of the leading pacemaker site may change due to conditions. We could not find the gradient of automaticity in *Arenicola* heart which suggests that automaticity potentially has a diffuse nature and that the whole heart could act as a pacemaker structure.

ACh had a strong positive chronotropic effect on the *Arenicola* heart, which was mediated by the increased slow diastolic depolarization rate. It is well known that ACh usually causes reduction of rhythm in the hearts of vertebrates as well as in most mollusks and some arthropods and that it usually has a stimulating effect on the neurogenic hearts (Prosser et al. 1951). It has been shown also that besides *Arenicola*, positive cardiotropic effects of ACh were found only in myogenic hearts of insects (Pitman 1971; McFarlane and Ting-Ya Fong 1972) and *Mytilidae* mollusks (Shigeto 1970). The latter studies have demonstrated that the effects of ACh in mussel myocardium are very similar to those that we observed in *Arenicola* heart. Shigeto (1970) noted that the ACh-induced acceleration of the rhythm correlates with increase in the diastolic depolarization rate and the reduction of maximal diastolic potential within the range of 2–13 mV. In several experiments, ACh caused complete cardiac arrest after a strong increase in beating rate and following membrane depolarization. However, Shigeto (1970) did not explore the receptor nature of positive ACh effects. In Hirudinea annelids, ACh causes depolarization of the cell membrane which can be effectively blocked by curare and mimicked by nicotinic agonists (Calabrese and Maranto 1986). Therefore, the effects of ACh in leech heart seem to be mediated by nicotinic-like receptors, which are pharmacologically similar to those on annelids body wall muscle and vertebrate autonomic ganglia (Calabrese and Maranto 1986). Our results indicate that the effects of ACh and its non-hydrolysable analog carbacholine in *Arenicola* heart are very sensitive to muscarinic antagonist atropine, but almost insensitive to high concentration of D-tubocurarine. Thus, the mechanisms of cholinergic effects are significantly different in Polychaeta and Hirudinea annelids.

The physiological role of ACh for the lugworm heart is also of interest. ACh usually acts as a neurotransmitter, but *Arenicola* heart does not have innervation. Therefore, we hypothesize that ACh could play a role as a humoral agent in *Arenicola* heart. Further experiments are required to locate the actual source of ACh in *Arenicola*'s heart and to understand the physiological mechanisms of cholinergic regulation on the *Arenicola*'s heart function in vivo.

Epinephrine ($10^{-8}$–$10^{-6}$ M) caused dose-dependent reduction of the rhythm in *Arenicola* heart up to the complete cessation of the pacemaker activity. It is known that among invertebrates, similar inhibitory effect of norepinephrine was observed only in the heart of the mussel (Wollemann and Rozsa 1975), although it was exerted by higher concentrations of norepinephrine ($10^{-6}$–$10^{-4}$ M). Wollemann and Rozsa (1975) demonstrated that these negative cardiotropic effects are mediated via inhibition of adenylate cyclase but they did not study a receptor

mechanism of norepinephrine action directly. Thus, *Arenicola marina* is found to be the second species that demonstrates cardioinhibitory effects in response to some adrenergic agonists. Moreover, the classical agonists and antagonists of mammalian $\alpha$ and $\beta$-adrenoreceptors, which are effective in the myocardium of vertebrates and several invertebrate species, did not affect *Arenicola* heart's function substantially. Therefore, we suggest that the myocardium of *Arenicola marina* expresses special type of adrenoreceptors that are pharmacologically different from the known vertebrate adrenoreceptor types.

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**Conflict of interest statement** The authors declare that they have no conflict of interest.

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