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SHORT COMMUNICATION

Diadenosine pentaphosphate affects electrical activity in guinea pig atrium via activation of potassium acetylcholine-dependent inward rectifier

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Abstract Diadenosine pentaphosphate (Ap5A) belongs to the family of diadenosine polyphosphates, endogenously produced compounds that affect vascular tone and cardiac performance when released from platelets. The previous findings indicate that Ap5A shortens action potentials (APs) in rat myocardium via activation of purine P2 receptors. The present study demonstrates alternative mechanism of Ap5A electrophysiological effects found in guinea pig myocardium. Ap5A (10^{-4} M) shortens APs in guinea pig working atrial myocardium and slows down pacemaker activity in the sinoatrial node. P1 receptors antagonist DPCPX (10^{-7} M) or selective GIRK channels blocker tertiapin (10^{-6} M) completely abolished all Ap5A effects, while P2 blocker PPADS (10^{-4} M) was ineffective. Patch-clamp experiments revealed potassium inward rectifier current activated by Ap5A in guinea pig atrial myocytes. The current was abolished by DPCPX or tertiapin and therefore was considered as potassium acetylcholine-dependent inward rectifier (I_{KACh}). Thus, unlike rat, in guinea pig atrium Ap5A produces activation of P1 receptors and subsequent opening of KACh channels leading to negative effects on cardiac electrical activity.

Keywords Heart · Atrium · Guinea pig · Action potential · Ionic current · I_{KACh} · Purine receptors · Diadenosine pentaphosphate

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Introduction

Diadenosine pentaphosphate (Ap5A) is one of the diadenosine polyphosphates (Ap(n)As) that have been recently considered as a new group of endogenous signaling compounds. Ap5A molecules consist of two adenosine residues connected with a chain of five phosphate residues (for review see [1]). Ap(n)As may act as neurotransmitters in central and peripheral nervous system [2], however the most well-known source of these compounds including Ap5A are activated platelets [3]. Thereby, cardiovascular system is the closest target for Ap(n)As.

Cardiovascular effects of Ap(n)As demonstrate great complexity depending on the animal species, the type of preparation and the tested compound. For example, Ap(n)As relax coronary arterial smooth muscle in pig [4], but produce marked constriction in human coronary arteries [5]. Therefore, the great diversity and contradictoriness of data obtained in isolated heart experiments with Ap(n)As (for review see [6]) is not surprising. The ability of Ap(n)As to influence cardiac electrical activity and even increase susceptibility of myocardium to arrhythmias [7] is beyond any doubt. However, the mechanisms of their electrophysiological effects are still poorly understood due to the relative lack of studies done at tissue or cellular level. Particularly, electrophysiological nature of Ap(n)As negative chronotropic effect, which was observed in isolated heart of rabbit [7] and guinea pig [8], has yet to be revealed.

The recent detailed study of electrophysiological effects produced by Ap5A and adenosine tetraphosphate (Ap4A) in different regions of rat heart showed principal similarity of response, shortening of action potentials (APs) in myocardium from right and left atrium, interatrial septum, right ventricular wall, and even myocardial sleeves of

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pulmonary veins [9]. AP shortening could be abolished by antagonists of P2 purine receptors, but was completely insensitive to P1 selective blocker DPCPX. In the present short communication, we demonstrate the principally different mechanism of Ap5A-induced AP shortening and slowing of sinoatrial node pacemaking activity. This mechanism includes activation of P1 receptors and coupled GIRK channels and works in guinea pig atrial myocardium, where the described P2-dependent mechanism seems to be absent.

Materials and methods

Experiments conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996). The experimental protocol was approved by the Bioethics Committee of Moscow State University. Male outbred guinea pigs weighing 270–310 g ($n = 23$) were held in the animal house for 4 weeks under a 12-h:12-h light:dark photoperiod in standard T4 cages prior to the experiment and fed ad libitum.

Guinea pigs were pretreated with heparin (1000 units/kg, i.p.) to prevent blood coagulation and anesthetized with a high dose of sodium pentobarbital (120 mg/kg). The chest was opened and the heart was rapidly excised and rinsed with Tyrode solution that contained (in mM): NaCl 118.0, KCl 2.7, NaH_2PO_4 2.2, $MgCl_2$ 1.2, $CaCl_2$ 1.8, $NaHCO_3$ 25.0, glucose 11.0, bubbled with carbogen gas (95% O_2 + 5% CO_2), pH 7.4. The right atrium was isolated and pinned endocardial side up to the bottom of the experimental chamber (3 ml) supplied with Tyrode solution at 10 ml min^{-1} (37.5 °C). Then two different types of preparations were obtained from the right atria. The first included the intercaval region (containing the sinoatrial node region) together with crista terminalis and was beating spontaneously. The second consisted of right auricle and was paced throughout the experiment with a pair of silver Teflon-coated electrodes (pacing rate—4 Hz, pulse duration—2 ms, pulse amplitude—2 times threshold).

After an hour of equilibration, transmembrane potentials were recorded from the endocardial surface of preparations with sharp glass microelectrodes (30–45 MΩ) filled with 3 M KCl connected to a high input impedance amplifier Model 1600 (A-M Systems, Sequim, WA, USA). The signal was digitized and analyzed using specific software (L-card, Russia; DI-Soft, Russia; Synaptosoft, USA). Stable impalements were maintained during the entire period of drug application. Changes in the resting potential and AP duration at 50% repolarization level (APD50) were measured in right auricle preparations. In preparation containing sinoatrial node changes in APD50, AP frequency (heart rate), maximal diastolic potential, and diastolic depolarization velocity were analyzed.

We used the previously described cell isolation procedure [10] with slight modifications. The hearts were isolated as described in the previous section. The hearts were attached to a Langendorff apparatus for retrograde perfusion with Ca^{2+}-free solution containing (in mM): NaCl 120, KCl 5.4, $MgSO_4 \cdot 7H_2O$ 5, Na-pyruvate 5, glucose 20, taurine 20, and HEPES 10 at pH of 6.9 adjusted with NaOH. After an initial perfusion period of 5 min with the Ca^{2+}-free solution, the hearts were perfused for 6–7 min with the same solution, supplemented with type II collagenase (0.16 mg ml^{-1}) and 20 μM $CaCl_2$. The perfusate was continuously bubbled with carbogen and the temperature was equilibrated at 37 °C. The right atrium was separated, chopped, and gently triturated to release the cells into standard Kraftbrühe medium [10]. The cells were stored in this medium for 5–6 h.

The whole-cell voltage clamp recording of K$^+$ inward rectifier currents (I_{Kir}) was performed using an EPC800-USB (HEKA Instruments, Germany) amplifier. The myocytes were superfused in a small recording chamber (RC-26; Warner Instrument Corp, Brunswick, CT, USA; volume 150 μl) mounted on an inverted microscope with solution containing (in mmol l^{-1}): 150 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose, 10 Hepes, with pH adjusted to 7.6 at 20 °C with NaOH at room temperature (24 ± 0.5 °C). Nifedipine (10^{-5} M), E-4031 (5×10^{-6} M) and chromanol 293B (5×10^{-5} M) [11] were added to extracellular solution in order to block I_{Ca}, I_{Kr}, and I_{Ks}, respectively. Patch pipettes with mean (± SEM) resistance of 2.86 ± 0.27 MΩ ($n = 66$) were pulled from borosilicate glass (Sutter Instrument, CA, USA) using PC-10 puller (Narishige, Japan) and filled with electrode solution containing (in mmol l^{-1}): KCl 140, $MgCl_2$ 1, EGTA 5, MgATP 4, MgGTP 0.03, and HEPES 10 with pH adjusted to 7.2 with KOH. Pipette capacitance was compensated after obtaining the seal with a resistance >2 GΩ. The whole cell capacitance and access resistance were completely compensated using the amplifier manual controls after getting access to the cell interior. The mean cell capacitance was 32.6 ± 4.4 pF, the mean access resistance was 8.3 ± 1.9 ($n = 23$). In order to obtain current densities, the peak currents were normalized by cell capacitance.

Nifedipine (blocker of Ca^{2+} channels), barium chloride (blocker of inward rectifier currents), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, selective antagonist of P1 purine receptors) and Ap5A were purchased from Sigma (St. Louis, MO, USA). E-4031 (I_{Kr} blocker), chromanol 293B (I_{Ks} blocker) and tertiapin (highly selective I_{KACh} blocker) were purchased from Tocris (Bristol, UK). Collagenase type II was purchased from Worthington (Lakewood, NJ, USA).

To exclude spontaneous degradation of Ap5A to AMP and adenosine, both dry substance and 10^{-2} M stock solutions of Ap5A were stored at -60 °C. Stock solutions were stored for not longer than 2 weeks and unfreezed right before preparation of working solution. Working solutions of Ap5A were applied to experimental chamber immediately after preparation.

All data in the text and figures except the original recordings are presented as mean $\pm$ SEM for n experiments. Significance of Ap5A effects on AP parameters and I_{Kir} was evaluated by paired t test. Mann–Whitney test or t test were used to compare effects of Ap5A in the absence and presence of blockers. $p < 0.05$ was adopted as the level of statistical significance. The normality of data arrays was checked using Shapiro–Wilk test.

Results

In paced preparations of guinea pig right atrium, three concentrations of Ap5A (10^{-6}, 10^{-5}, and 10^{-4} M) were tested. In control conditions, the average APD50 was 35.7 ± 2.3 ms ($n = 16$). While 10^{-6} M Ap5A was completely ineffective (data not shown), 10^{-5} M Ap5A tended to decrease APD50, although these changes were not statistically significant (Fig. 1c). 10^{-4} M Ap5A produced marked AP shortening (Fig. 1) by $33.2 \pm 6.6\%$ of control APD50 ($n = 12$, $p < 0.05$). The effect developed rapidly, reaching the maximal level in 2 min after the start of Ap5A application. No substantial changes in resting membrane potential were observed.

To reveal the mechanisms of Ap5A-induced AP shortening, the selective antagonist of P1 purine receptors DPCPX, antagonist of P2 receptors PPADS, or highly selective blocker of acetylcholine-dependent potassium channels (KACh) tertiapin were added to the experimental chamber after 5 min of pure Ap5A application. Both DPCPX (10^{-7} M) and tertiapin (10^{-6} M) completely abolished Ap5A-induced AP shortening (Fig. 1). The AP waveform after addition of blockers in the presence of Ap5A did not differ significantly from the control. PPADS (10^{-4} M) failed to alter the effect of Ap5A (Fig. 1c, d). Also, prior pilot experiments with PPADS showed that the 10^{-4} M of blocker has none of its own effects on electrical activity in guinea pig atrium.

In the experiments with sinoatrial node preparations, we have registered electrical activity of primary pacemaker type, which is characterized by a smooth transition from diastolic depolarization to the upstroke of AP and relatively low velocity of AP upstroke [12]. Similarly to working atrial myocardium, 10^{-6} M Ap5A was ineffective in the pacemaker, while 10^{-5} M Ap5A tended to shorten APs and decelerate the rhythm (Fig. 2d, e). Only 10^{-4} M Ap5A

produced significant slowing of sinus rhythm (Fig. 2a–c, e), which was accompanied by AP shortening at 50% repolarization level (Fig. 2d), reduction of diastolic depolarization velocity (Fig. 2g) and hyperpolarization, which was estimated as negative shift of maximal diastolic potential (Fig. 2f). Tertiapin (10^{-6} M) abolished all mentioned effects of Ap5A on pacemaking electrical activity (Fig. 2b, d–g). DPCPX (10^{-7} M) was almost similarly effective (Fig. 2a, d, e, g), but failed to produce statistically significant block of Ap5A-induced hyperpolarization (Fig. 2f). PPADS (10^{-4} M) was completely ineffective (Fig. 2c–g).

Since tertiapin, which is highly specific for KACh channels [13], completely blocked Ap5A effects in both working and pacemaking guinea pig myocardium, we hypothesized that activation of these channels underlie the observed effects. To test this hypothesis, patch-clamp recording of I_{Kir} currents was conducted in isolated guinea pig atrial myocytes.

The family of mammalian cardiac inward rectifiers consists of three distinct currents: (1) the background inward rectifier current (I_{K1}), (2) the acetylcholine-dependent inward rectifier (I_{KACh}) and (3) the ATP-sensitive current (I_{KATP}) [14]. However, I_{KACh} and I_{KATP} are absent in normal conditions, when the muscarinic receptors are not stimulated and intracellular ATP content is not too low. Therefore, in basal conditions, we recorded only I_{K1} with strong inward component at potentials more negative than K^+ equilibrium potential. The outward component of that current had a maximum at around -60 mV and was completely absent at potentials more positive than -20 mV (Fig. 3).

In 14 of 16 tested myocytes, application of 10^{-4} M Ap5A activated additional current, which had not only substantial inward component, but an outward component, which persisted at 0 mV and even at positive potentials (Fig. 3a, d). This current obviously belongs to the inward rectifier family, because almost all other prominent currents were blocked in our protocol; moreover, 1 mM BaCl$_2$t could completely block it together with I_{K1}. After BaCl$_2$ application, only the leakage current was registered. The Ap5A-induced current demonstrated current–voltage dependence typical for I_{KACh}. Since this current was completely blocked by 10^{-6} M tertiapin (Fig. 3a, c, d), very selective for KACh channels, we conclude that Ap5A in fact activates I_{KACh}. Note that tertiapin did not affect I_{K1}. This current could also be completely abolished by the addition of P1 blocker 10^{-7} M DPCPX (Fig. 3b–d). In a few pilot experiments with P2 blocker PPADS, we observed complete absence of its effect on Ap5A-induced I_{KACh} (data not shown). Thus, P1 purine receptors mediate I_{KACh} activation by Ap5A.
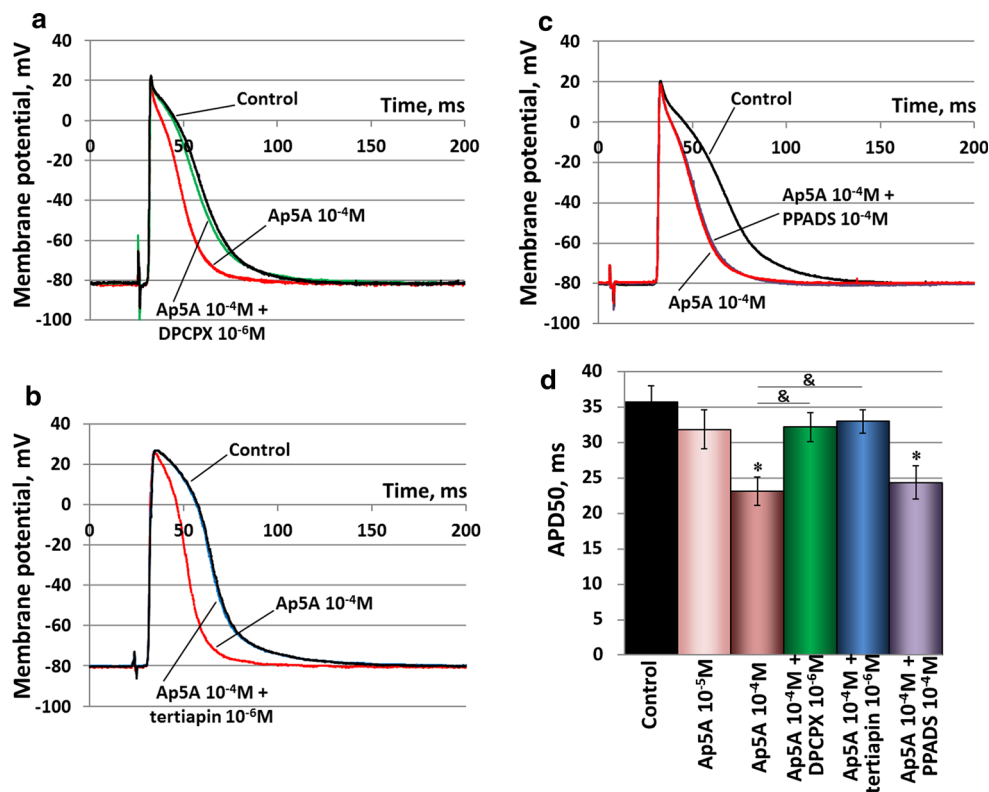
Fig. 1 Effects of Ap5A on the action potential (AP) waveform in the working myocardium of guinea pig right atrium. **a–c** Original recordings of APs in three different representative preparations of guinea pig right auricle. APs recorded in control conditions, under the action of Ap5A (10^{-4} M) alone or together with P1 blocker DPCPX (**a**), I_{KACh} blocker tertiapin (**b**), or P2 blocker PPADS (**c**) are superimposed. **d** Comparison of mean ($\pm$SEM) values of AP duration at 50% repolarization level in control conditions ($n = 16$), in the presence of Ap5A (10^{-5} M $n = 5$, 10^{-4} M $n = 12$) alone or together with DPCPX ($n = 6$), tertiapin ($n = 6$) or PPADS ($n = 6$). An *asterisk* indicates a statistically significant difference ($p < 0.05$, paired t test) from the control value. *Ampersand* Significant difference between the columns ($p < 0.05$, Mann–Whitney test)

Discussion

This is the first investigation, to our knowledge, of Ap5A's effects on the electrical activity of working atrial myocardium and sinoatrial node of guinea pig. Although the negative chronotropic effect of Ap5A in the isolated guinea pig heart was first noticed by Brandts et al. [8] almost two decades ago, the electrophysiological nature of Ap5A-induced rhythm slowing has never been studied in guinea pig or any other mammal. Here we demonstrate that Ap5A, at least in high concentration (10^{-4} M), produces the marked slowing of diastolic depolarization which, together with negative shift of maximal diastolic potential, leads to bradycardia. This mechanism is very similar to the well-known bradycardic effect of acetylcholine [15] and therefore the strong block of Ap5A effect by tertiapin is not surprising.

The Ap5A-induced changes in AP waveform have never been shown in guinea pig atrial muscle. In ventricular tissue, both nanomolar [16] and micromolar [17] concentrations of Ap5A were shown to increase AP duration. Taking into account the shortening of atrial APs by Ap5A

[9] observed in another species (rat), such discrepancy between response to Ap5A in atrial and ventricular myocardium of guinea pig seems to be not a big trouble. Ventricular muscle greatly differs from the atrial by expression level of various ionic channels and receptors. For example, in guinea pig, the sensitivity to acetylcholine is many times greater in atrium than in ventricle [18].

In the present study, we provide clear evidence of I_{KACh} and P1 receptor involvement in mediation of Ap5A effects in the atrial myocardium. P1 receptors are coupled with G_i proteins, which activate GIRK channels responsible for I_{KACh} via βγ-subunit [19]. The activation of I_{KACh} by typical P1 agonist, adenosine, has been reported in atrial myocytes from several mammalian species [19, 20] and is known to produce electrophysiological effects similar to what we have shown for Ap5A. The fact that both I_{KACh} activation by Ap5A and changes in electrical activity induced by this compound could be blocked by either tertiapin or DPCPX proves the hypothesis that P1 receptors mediate Ap5A's effects similarly to adenosine effects in guinea pig atrium. The completeness of this block together with high selectivity of tertiapin, peptide blocker isolated

Fig. 2 Effects of Ap5A on the electrical activity of guinea pig sinoatrial node (SAN). **a–c** Original traces of electrical activity recorded in the central part of SAN in three different representative preparations. Traces recorded in control conditions, under the action of Ap5A (10^{-4} M) alone or together with P1 blocker DPCPX (**a**), I_{KACh} blocker tertiapin (**b**) or P2 blocker PPADS (**c**) are superimposed. **d–g** Comparison of various parameters of pacemaker electrical activity in control conditions ($n = 11$), in the presence of Ap5A (10^{-5} M $n = 5$, 10^{-4} M $n = 11$) alone or together with DPCPX ($n = 6$), tertiapin ($n = 5$) or PPADS ($n = 5$). An *asterisk* indicates a statistically significant difference ($p < 0.05$, paired *t* test in **d**, **e**, **g**; Wilcoxon test in **g**) from the control value. *Ampersand* Significant difference between the columns ($p < 0.05$, *t* test in **d**, **e**; Mann–Whitney test in **f**, **g**)
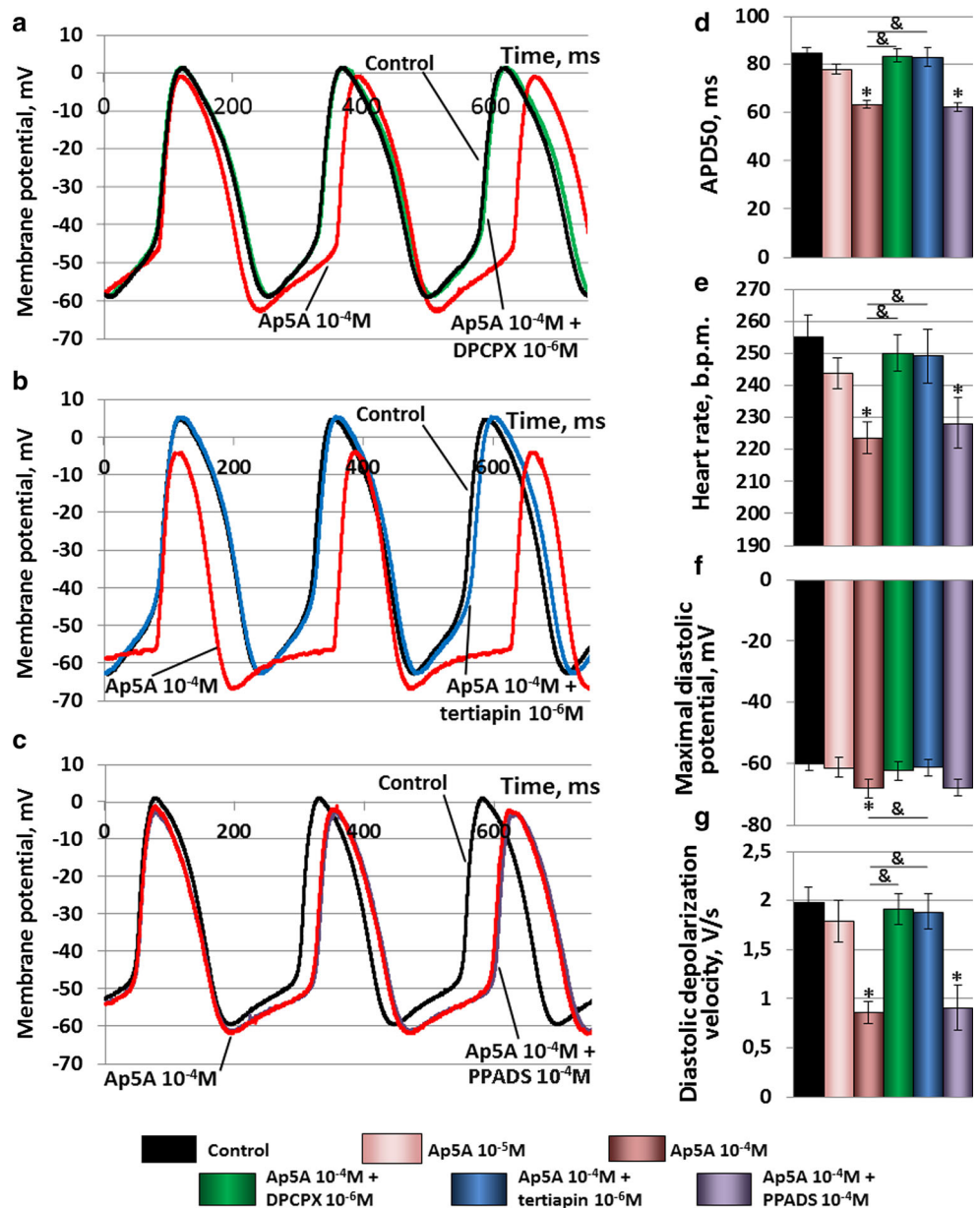
from bee venom [13], make us sure that no other type of receptors participate in mediation of the described Ap5A effects. Also, DPCPX is known as a selective P1 antagonist [21]. In rat myocardium, where Ap(n)A's effects are P2-dependent, DPCPX failed to attenuate Ap5A-induced AP shortening even slightly [9]. At the same time, P2 antagonist PPADS taken in quite high concentration (10^{-4} M) completely failed to alter the intensity of Ap5A's effects in both the working atrial myocardium and pacemaker of guinea pig heart. In rat, where the effects of Ap(n)A are P2-dependent, 10^{-4} M PPADS completely abolished them [9]. Such great difference between the influence of DPCPX and PPADS in guinea pig and rat atrial myocardium allows to conclude that P2 receptors do not participate in realization

of Ap5A-dependent cardiac electrophysiological effects in guinea pig.

At the same time, the presence of P2 receptors in guinea pig atrium cannot be denied, since Hoyle et al. [22] have demonstrated their involvement in the negative inotropic response to Ap5A. The same study claims Ap(n)A activation of P1 receptors as another mechanism of negative inotropy. Our data supports and explains this conclusion, since shortening of AP obviously leads to suppression of contractile activity. However, unlike the effects on cardiac contractility, electrophysiological effects of Ap5As are completely P1-dependent.

It is well known that soluble and membrane-binded ectohydrolases and ectonucleotidases (NT5 or CD73,
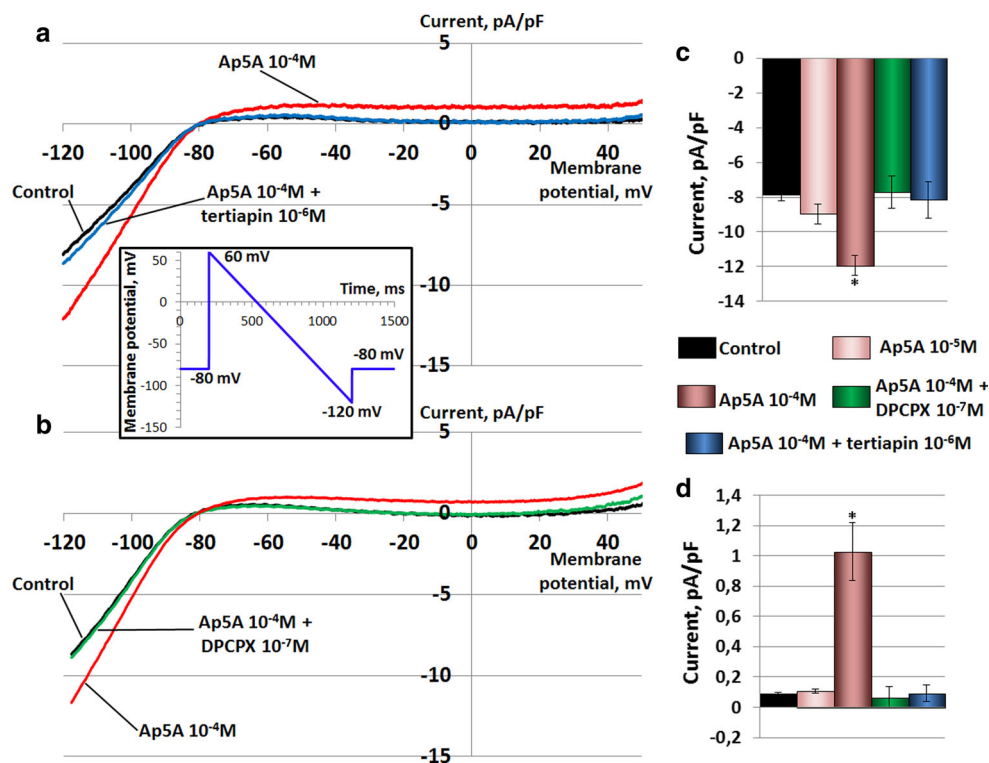
Fig. 3 Effects of Ap5A on the total inward rectifier K^+ current (I_{Kir}, including the background I_{K1} and the I_{KACh}) in atrial myocytes of the guinea pig heart. **a, b** Original current–voltage curves of I_{Kir} recorded in two different representative atrial myocytes in control conditions, during application of 10^{-4} M Ap5A and after subsequent application of **a** I_{KACh} blocker tertiapin (10^{-6} M) or **b** antagonist of P1 receptors DPCPX (10^{-7} M). The current was elicited by hyperpolarizing voltage ramp shown in the inset. The current–voltage curves were built after subtraction of leakage current recorded after addition of 1 mM $BaCl_2$ in the end of each experiment (**c, d**). Comparison of mean ($\pm$SEM) values of I_{Kir} measured at -120 mV (**c**) and 0 mV (**d**) in control conditions ($n = 14$), in the presence of Ap5A (10^{-5} M $n = 7$, 10^{-4} M $n = 14$) alone or together with DPCPX ($n = 7$) or tertiapin ($n = 7$). An *asterisk* indicates a statistically significant difference ($p < 0.05$, paired t test) from the control value

CD39, CD203) may hydrolyze such nucleotides as NAD, ATP, and ADP down to AMP, adenosine and inorganic phosphates. Extracellular degradation of dinucleotide polyphosphates has been also shown in various tissues. The central role in hydrolysis of Ap(n)As belongs to pyrophosphatases/phosphodiesterases [23]. In guinea pig heart, activation of P1 purine receptors by Ap(n)As-derived adenosine has been suggested as a mechanism of Ap(n)As action [8]. In the same study, Ap5A, unlike adenosine, failed to induce I_{KACh} in isolated guinea pig atrial cells, allowing to conclude that P1-mediated Ap(n)As effects are in fact produced by adenosine, the product of Ap(n)As degradation. Our findings directly argue this conclusion, since Ap5A was shown to activate I_{KACh} via P1 receptors stimulation in isolated atrial myocytes, where tissue pyrophosphatases/phosphodiesterases are not present. The possible reason for this discrepancy between two studies may be due to the different state of myocytes after enzymatic isolation. Longer enzymatic digestion can lead to degradation of membrane receptors, including the P1 receptors, and abolish the normal response to Ap5A. In our experiments, two myocytes also demonstrated complete insensitivity even to the highest Ap5A concentration. Possible presence of adenosine in our Ap5A solutions due to spontaneous Ap5A degradation was excluded by using special conditions of Ap5A storage and dilution (see Materials and methods section).

The physiological significance of presented findings might seem questionable since quite-high micromolar concentrations of Ap5A were used. However, despite low concentrations of Ap(n)As in blood serum, their local level in myocardium may reach high amounts. For example, Luo et al. [24] have estimated concentrations of Ap(n)As including Ap5A in myocardial secretory chromaffin granules from human heart. They appeared to be in the range of 0.5 mM. Thus, we suppose that Ap5A concentrations used in the present study might be physiologically relevant, although exact estimation of extracellular Ap5A concentrations is a goal for future investigations.

Thus, in guinea pig atrial myocardium, Ap5A produces negative effects (AP shortening, sinus rhythm deceleration) via stimulation of P1 receptors and subsequent I_{KACh} induction. This mechanism of Ap(n)As effects on cardiac electrical activity has not been described in mammals and

is alternative to P2-dependent effects of Ap(n)As found in rat heart. Our findings demonstrate striking species-specificity of Ap(n)As cardioregulatory effects.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

References

1. Delicado EG, Miras-Portugal MT, Carrasquero LM, León D, Pérez-Sen R, Gualix J (2006) Dinucleoside polyphosphates and their interaction with other nucleotide signaling pathways. Pflugers Arch 452:563–572
2. Mutafova-Yambolieva VN, Durnin L (2014) The purinergic neurotransmitter revisited: a single substance or multiple players? Pharmacol Ther 144:162–191
3. Schlüter H, Offers E, Brüggemann G, van der Giet M, Tepel M, Nordhoff E, Karas M, Spieker C, Witzel H, Zidek W (1994) Diadenosine phosphates and the physiological control of blood pressure. Nature 367:186–188
4. Sumiyoshi R, Nishimura J, Kawasaki J, Kobayashi S, Takahashi S, Kanaide H (1997) Diadenosine polyphosphates directly relax porcine coronary arterial smooth muscle. J Pharmacol Exp Ther 283:548–556
5. Conant AR, Theologou T, Dihmis WC, Simpson AW (2008) Diadenosine polyphosphates are selective vasoconstrictors in human coronary artery bypass grafts. Vascul Pharmacol 48:157–164
6. Stavrou BM (2003) Diadenosine polyphosphates: postulated mechanisms mediating the cardiac effects. Curr Med Chem Cardiovasc Hematol Agents 1:151–169
7. Brandts B, Borchard R, Dirkmann D, Wickenbrock I, Sievers B, van Bracht M, Prull MW, Trappe HJ (2003) Diadenosine-5-phosphate exerts A1-receptor-mediated proarrhythmic effects in rabbit atrial myocardium. Br J Pharmacol 139:1265–1272
8. Brandts B, Brandts A, Wellner-Kienitz MC, Zidek W, Schluter H, Pott L (1998) Non-receptor-mediated activation of IK(ATP) and inhibition of IK(ACh) by diadenosine polyphosphates in guinea-pig atrial myocytes. J Physiol 512:407–420
9. Pustovit KB, Kuzmin VS, Abramochkin DV (2016) Diadenosine tetra- and pentaphosphates affect contractility and bioelectrical activity in the rat heart via P2 purinergic receptors. Naunyn Schmiedebergs Arch Pharmacol 289:303–313
10. Isenberg G, Klockner U (1982) Calcium-tolerant ventricular myocytes prepared by preincubation in a 'KB-medium'. Pflugers Arch 395:6–18
11. Jing L, Brownson K, Patwardhan A (2014) Role of slow delayed rectifying potassium current in dynamics of repolarization and electrical memory in swine ventricles. J Physiol Sci 64:185–193
12. Bleeker WK, Mackaay AJC, Masson-Pevet M, Bouman LN, Becker AE (1980) Functional and morphological organization of the rabbit sinus node. Circ Res 46:11–22
13. Kitamura H, Yokoyama M, Akita H, Matsushita K, Kurachi Y, Yamada M (2000) Tertiapin potently and selectively blocks muscarinic K(+) channels in rabbit cardiac myocytes. J Pharmacol Exp Ther 293:196–205
14. Hibino H, Inabone A, Furutani K (2010) Inwardly rectifying potassium channels: their structure, function, and physiological roles. Physiol Rev 90:291–366
15. Boyett MR, 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
16. Stavrou BM, Lawrence C, Blackburn GM, Cohen T, Sheridan DJ, Flores NA (2001) Coronary vasomotor and cardiac electrophysiologic effects of diadenosine polyphosphates and nonhydrolyzable analogs in the guinea pig. J Cardiovasc Pharmacol 37:571–584
17. Arvola L, Bertelsen G, Hassaf D, Ytrehus K (2004) Positive inotropic and sustained anti-beta-adrenergic effect of diadenosine pentaphosphate in human and guinea pig hearts. Role of dinucleotide receptors and adenosine receptors. Acta Physiol 182:277–285
18. Zang WJ, Chen LN, Yu XJ, Fang P, Lu J, Sun Q (2005) Comparison of effects of acetylcholine on electromechanical characteristics in guinea-pig atrium and ventricle. Exp Physiol 90:123–130
19. Ragazzi E, Wu SN, Shryock J, Belardinelli L (1991) Electrophysiological and receptor binding studies to assess activation of the cardiac adenosine receptor by adenine nucleotides. Circ Res 68:1035–1044
20. Lomax AE, Rose RA, Giles WR (2003) Electrophysiological evidence for a gradient of G protein-gated K + current in adult mouse atria. Br J Pharmacol 140:576–584
21. Collis MG, Stogall SM, Martin FM (1989) Apparent affinity of 1,3-dipropyl-8-cyclopentylxanthine for adenosine A1 and A2 receptors in isolated tissues from guinea-pigs. Br J Pharmacol 97:1274–1278
22. Hoyle CH, Ziganshin AU, Pintor J, Burnstock G (1996) The activation of P1- and P2-purinoceptors in the guinea-pig left atrium by diadenosine polyphosphates. Br J Pharmacol 118:1294–1300
23. Vollmayer P, Clair T, Goding JW, Sano K, Servos J, Zimmermann H (2003) Hydrolysis of diadenosine polyphosphates by nucleotide pyrophosphatases/phosphodiesterases. Eur J Biochem 270(14):2971–2978
24. Luo J, Jankowski V, Gungar N, Neumann J, Schmitz W, Zidek W, Schluter H, Jankowski J (2004) Endogenous diadenosine tetraphosphate, diadenosine pentaphosphate, and diadenosine hexaphosphate in human myocardial tissue. Hypertension 43(5):1055–1059