PHOSPHOLIPASE C INHIBITOR PREVENTS THE EFFECTS OF METHOXAMINE ON THE ACTION POTENTIAL OF CARDIOMYOCYTES IN RATS OF DIFFERENT AGES

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Abstract. In the present study, we investigated the effect of α1-adrenergic receptor stimulation on the electrical activity of the right atrium cardiomyocytes with imposed rhythm in rats of different ages using methoxamine and methoxamine against the background of selective blockade of phospholipase C inhibitor (U-73122). Methoxamine increased the duration of the repolarization phase of the action potential. However, there were no changes in the other electrophysiological parameters studied. U-73122 markedly blocked all effects of α1-adrenoreceptor stimulation on the parameters of the electrical activity of working cardiomyocytes in 7-, 21-, and 100-day-old rats.

Keywords: U-73122, methoxamine, action potential duration, heart.

List of Abbreviations

AR – Adrenergic receptor
APD – action potential duration
PLC – Phospholipase C
PIP_2 – phosphatidylinositol 4,5-bisphosphate
IP_3 – inositol 1,4,5-triphosphate
DAG – diacylglycerol
PKC– Protein Kinase C
MAPK – Mitogen-Activated Protein Kinase
PI_3K – Phosphatidylinositol 3-Kinase

Introduction

The transmembrane proteins known as alpha-1 adrenergic receptors have an extracellular N-terminal domain and seven helical domains. Stimulation of α1-adrenoceptors has been reported to have acute effects, such as rapid regulatory changes in the contractile function and electrophysiological properties of cardiac muscle cells. It also has long-term effects on cardiac structure and function, including cellular hypertrophy, gene transcription, protein synthesis, and regulation of apoptosis (Woodcock, 2007; Ichishima *et al.*, 2010).

There are three subtypes of alpha-1 adrenergic receptors: alpha-1A, alpha-1B, and alpha-1D, each with distinct tissue distributions and physiological functions. Activation of alpha-1 adrenergic receptors by agonists, such as norepinephrine, initiates several intracellular signalling pathways that involve coupling to G proteins of the Gq/11 family, followed by activation of a phospholipase Cβ (PLCβ). This activation leads to the cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), thereby triggering the PLC-IP_3 pathway (Hein & Michel, 2007). The calcium-sensitive enzyme protein kinase C (PKC) is activated when IP_3 binds to its receptor on the endoplasmic reticulum, causing the release of Ca^{2+} into the cytoplasm. Several transcription factors that control gene expression are activated by the PKC pathway. The control of cell division and proliferation is facilitated by the MAPK pathway, while cell survival and apoptosis are regulated by the PI_3K pathway (O'connell *et al.*, 2013; Bogoyevitch *et al.*, 1996).

In recent years, substantial advancements have been made in understanding the molecular properties and functional implications of the α1-adrenergic receptor (α1-AR) subtypes, derived from both in vitro and in vivo studies. While numerous investigations have concentrated on individual receptor subtypes, only a limited number have sought to compare the behaviors of different receptors under similar experimental conditions (Cotecchia, 2011). Recent research has highlighted the pivotal role of α1-adrenergic receptors in the maintenance of cardiac function. Although α1-ARs are less

prevalent in the heart compared to β1-adrenergic receptors, the activation of cardiac α1-ARs triggers significant biological processes. The nonselective blockade of α1-ARs is associated with a twofold increase in adverse cardiac events, including heart failure and angina, suggesting that α1-AR activation may confer cardioprotective benefits in humans. This observation underscores a potential cardioprotective role for the stimulation of α1A-ARs, thus indicating a shift in paradigm from solely adrenergic blockade to a more comprehensive approach of adrenergic modulation (Zhang *et al.*, 2021).

Methoxamine is a well-known α1-adrenoceptor agonist, clinically used as a longer-acting analogue of epinephrine (Kohutova *et al.*, 2023). The stimulation of α1-adrenoreceptors by methoxamine in rats results in a decreased contraction rate of the isolated adult rat heart. Furthermore, the intravenous administration of methoxamine induces cardiac bradycardia throughout the organism (Zefirov *et al.*, 2016). In newborn rats, stimulation of α1-adrenergic receptors, irrespective of methoxamine concentration, leads to a negative inotropic response in both the atrial and ventricular myocardium (Khabibrakhmanov *et al.*, 2020).

The activation of α1-adrenergic receptors by methoxamine also significantly affects the studied electrophysiological parameters. In newborn rats, the α1-adrenergic agonist methoxamine increased the duration of the repolarization phase of the action potential under both imposed and intrinsic rhythms (Mansour *et al.*, 2023). Conversely, in adult rats, methoxamine exhibited a dual effect on repolarization duration; it increased the duration with an imposed rhythm while decreasing it in the presence of intrinsic rhythms (Mansour *et al.*, 2023).

Increasing evidence suggests that the diverse functional effects mediated by α1-ARs across different organs likely indicate the activation of multiple signaling pathways, particularly through phospholipase C (PLC) via Gq/11 signaling. The initial discovery of U-73122 as a PLC inhibitor by Bleasdale, Smith, and colleagues (Bleasdale *et al.*, 1990; Smith *et al.*, 1990) provided a valuable tool for assessing the contribution of PLC to cellular signaling pathways in various cell types (Hollywood *et al.*, 2010). Substantial progress has been made in elucidating the molecular mechanisms underlying G protein-dependent PLC activation; however, further investigations are necessary to comprehensively clarify these mechanisms (Chandan *et al.*, 2022).

The adrenergic regulation of a newborn rat's heart features immature sympathetic innervation, in contrast to that of an adult rat. At 21 days old, rats exhibit the onset of adrenergic innervation of the heart, accompanied by an increase in heart rate. Therefore, studies on animals of different ages are of particular interest (Ziyatdinova *et al.*, 2019; Khabibrakhmanov *et al.*, 2020).

While previous research has focused on α1-adrenergic receptor stimulation in adult or newborn rats, our study investigates these effects across different developmental stages. We consider these age groups in relation to the development of sympathetic innervation. Although the role of α1-adrenergic receptors is well-known, the involvement of phospholipase C (PLC) in these effects remains less clear. Studying methoxamine in the presence of U-73122 could help elucidate the importance of PLC in modulating the electrical activity of cardiomyocytes in rats of different ages upon stimulation of α1-adrenergic receptors, thereby offering a deeper understanding of the intracellular pathways involved. This knowledge is vital for developing age-specific therapies and understanding heart development.

Our study aimed to investigate the role of phospholipase C in the effects caused by selective stimulation of α1-adrenergic receptors on the parameters of myocardial electrical activity in rats of different ages with imposed rhythm.

Materials and Methods

The study was conducted on 7-day-old (newborn; n = 20), 21-day-old (n = 20) and 100-day-old (adult; n = 15) albino rats using microelectrode technology. The experimental protocol was approved by the Ethics Committee of Kazan Federal University (Protocol No. 39 of December 22, 2022). After anesthesia with ure-

thane (25%), the chest was opened, and the heart was excised and transferred to a Petri dish. An isolated right atrial myocardium with a preserved sinus node and spontaneous activity was prepared. During the experiment, the right atrial preparation was continuously perfused with a solution containing (in mM): NaCl, 133.47; KCl, 4.69; NaH2PO4.2H2O, 1.35; NaHCO3, 16.31; MgSO4.7H2O, 1.18; CaCl2.2H2O, 2.5; glucose, 7.77, and saturated with carbogen (95% O2, 5% CO2), at pH 7.2 –7.4 and a temperature of 37±1 ºC. The experiments were performed under imposed rhythm; atria were stimulated using platinum electrodes at a rate of 150 beats per minute. Intracellular action potentials were recorded via glass microelectrodes with a resistance of 25–60 MΩ. After a waiting period of 35–40 minutes for the preparation to adapt, control signals were recorded. Subsequently, the alpha-1-adrenergic agonist methoxamine (Tocris) was dissolved in the working solution at a concentration of 10^{-8} M for data registration. To investigate the role of phospholipase C (PLC) in the effects caused by stimulation of α1-adrenergic receptors with methoxamine, experiments were conducted using the phospholipase C inhibitor: U-73122 (10 μM). U-73122 (Tocris) was initially dissolved in DMSO as a stock solution and then diluted to achieve the final concentration in the working solution. To study the stimulation of alpha1-adrenergic receptors by methoxamine in the presence of U-73122 blockade, control signals were recorded first, followed by the addition of U-73122 (10 μM) to the working solution. After 20 minutes, methoxamine (10^{-8} M) was added. The concentrations used were based on previous experiments. Signals were recorded using the Elph 3.0 software. The parameters measured included the membrane potential, action potential, duration of depolarization, amplitude of the action potential, and duration of the action potential at 20% (APD 20%), 50% (APD 50%), and 90% (APD 90%) of the repolarization phase. Statistical significance was assessed using one-way ANOVA. The results of the experiments are expressed as mean ± standard error of the mean, with differences considered statistically significant at $p < 0.05$.

Results

Effect of α1-adrenergic receptor stimulation with methoxamine and methoxamine in the presence of U-73122 on the parameters of the electrical activity of working cardiomyocytes in 7-day-old rats with imposed rhythm: Methoxamine at a concentration of 10^{-8} M (n = 10) increased the duration of the action potential at APD 20%, APD 50%, and APD 90% by 56.9% ($p < 0.01$), 53.8% ($p < 0.01$), and 40.3% ($p < 0.01$), respectively, while the duration of depolarization phase did not change. The values of the amplitude of the action potential and the membrane potential did not change.

The next series of experiments aimed to study the stimulation of α1-adrenergic receptors with methoxamine in the presence of U-73122 on the parameters of the electrical activity of working cardiomyocytes in newborn rats with imposed rhythm (n = 10). U-73122 (10 μM) alone did not have a significant effect on the studied electrophysiological parameters. In the presence of U-73122 (10 μM), methoxamine (10^{-8} M) did not change the membrane potential, the amplitude of the action potential, the duration of the depolarization phase, or the duration of the action potential at APD 20%, APD 50%, or APD 90% of the repolarization phase (Fig. 1, 2).

Effect of α1-adrenergic receptor stimulation with methoxamine and methoxamine in the presence of U-73122 on the parameters of the electrical activity of working cardiomyocytes in 21-day-old rats with imposed rhythm:
Methoxamine at a concentration of 10^{-8} M (n = 10) increased the duration of the action potential at APD 20%, APD 50%, and APD 90% by 56.5% ($p < 0.01$), 54.5% ($p < 0.01$), and 36.8% ($p < 0.01$), respectively, while the duration of the depolarization phase did not change. The amplitude of the action potential and the membrane potential also did not change. U-73122 (10 μM) alone did not have a significant effect on the studied electrophysiological parameters of working cardiomyocytes in 21-day-old rats with imposed rhythm. In the presence of U-73122 (10 μM), methoxamine (10^{-8} M) (n = 10) in 21-day-old rats did not change the membrane potential, the amplitude

of the action potential, the duration of the depolarization phase, or the duration of the action potential at APD 20%, APD 50%, or APD 90% of the repolarization phase (Fig. 3, 4).

Effect of α1-adrenergic receptor stimulation with methoxamine and methoxamine in the presence of U-73122 on the parameters of the electrical activity of working cardiomyocytes in 100-day-old rats with imposed rhythm:

Methoxamine at a concentration of 10^{-8} M (n = = 8) increased the duration of the action potential at APD 20%, APD 50%, and APD 90% by 20.5% ($p < 0.01$), 23.9% ($p < 0.01$), and 8.2% ($p < 0.01$), respectively, while the duration of the depolarization phase did not change. The values of the amplitude of the action potential and membrane potential also did not change.

U-73122 (10 μM) alone did not have a significant effect on the studied electrophysiological parameters of working cardiomyocytes in 100-day-old rats with imposed rhythm. In the presence of U-73122 (10 μM), methoxamine (10^{-8} M) (n = 7) in 100-day-old rats did not alter the membrane potential, the amplitude of the action potential, the duration of the depolarization phase, or the duration of the action potential at APD 20%, APD 50%, or APD 90% of the repolarization phase (Fig. 5, 6).

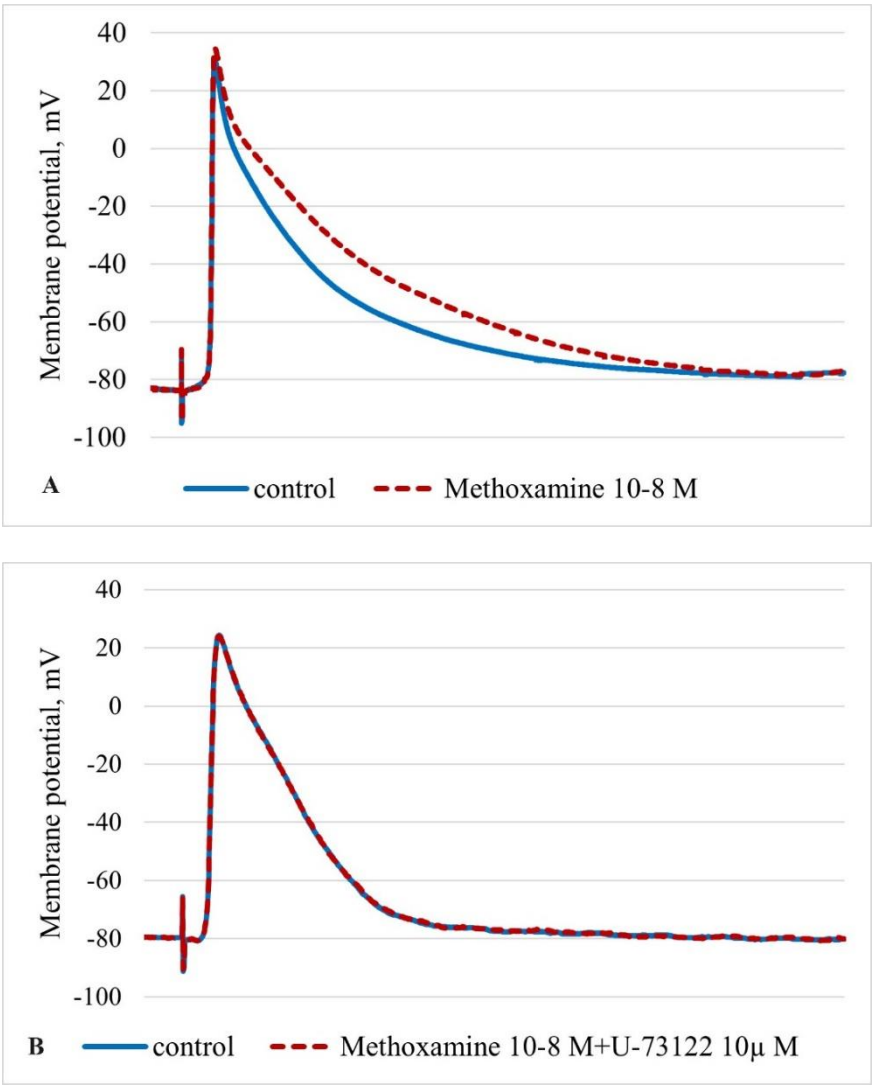

Fig.1. Original recordings of action potentials (AP) of working cardiomyocytes in the right atrium of 7-day-old rats under imposed rhythm upon stimulation of α1-adrenoceptors with methoxamine 10^{-8} M (A) and in the presence of PLC blockade with U-73122 (10 μM) (B)

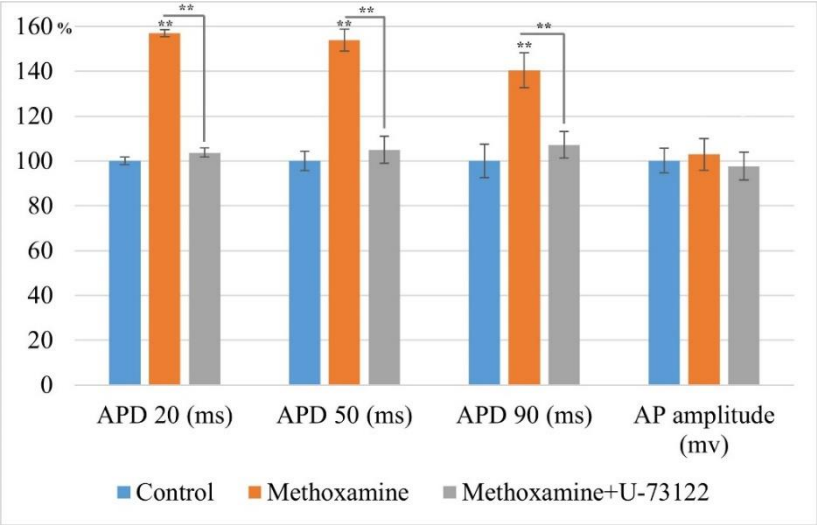

Fig. 2. Percentage effects of methoxamine (10^{-8} M) (n = 10) and methoxamine (10^{-8} M) + U-73122 (10μ M) (n = 10) on amplitude-time parameters in 7-day-old rats under imposed rhythm (note *- p < 0.05, **- p < 0.01)

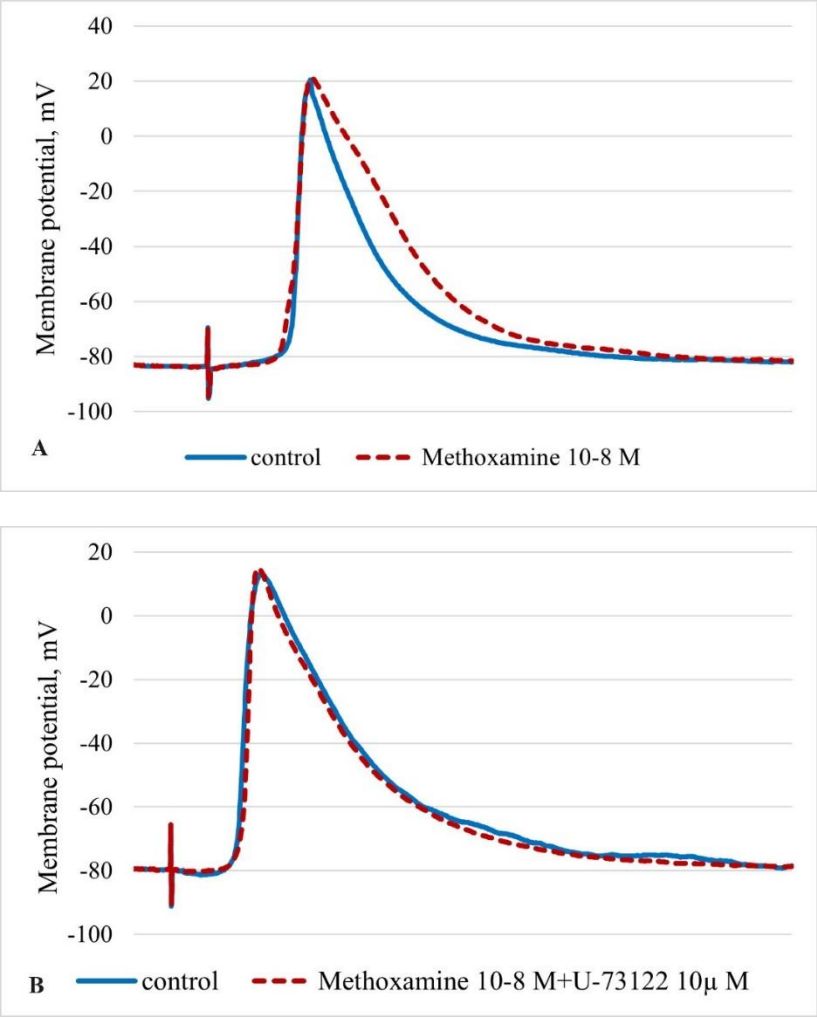

Fig. 3. Original recordings of action potentials (AP) of working cardiomyocytes in the right atrium of 21-day-old rats under imposed rhythm upon stimulation of α1-adrenoceptors with methoxamine 10^{-8} M (A) and in the presence of PLC blockade with U-73122 (10 μM) (B)

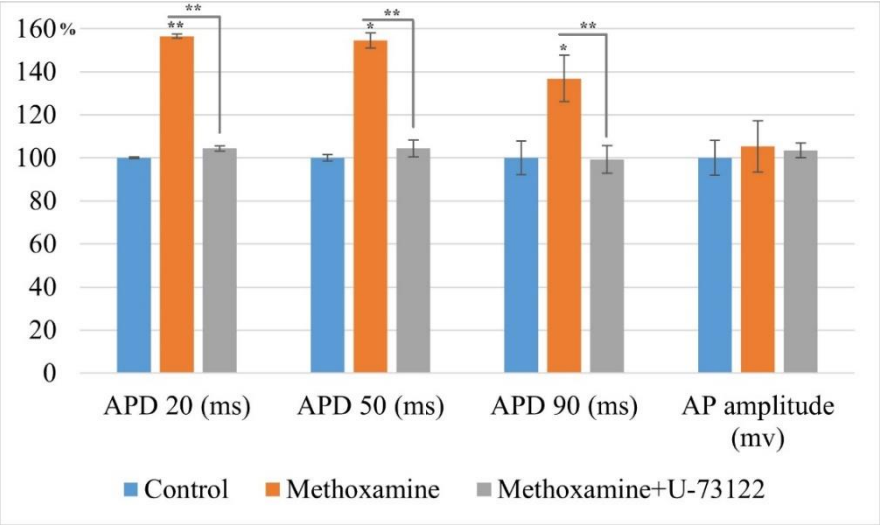

Fig.4. Percentage effects of methoxamine (10^{-8} M) (n = 10) and methoxamine (10^{-8} M) + U-73122 (10 μM) (n = = 10) on amplitude-time parameters in 21-day-old rats under imposed rhythm (note *- p < 0.05, **- p < 0.01)

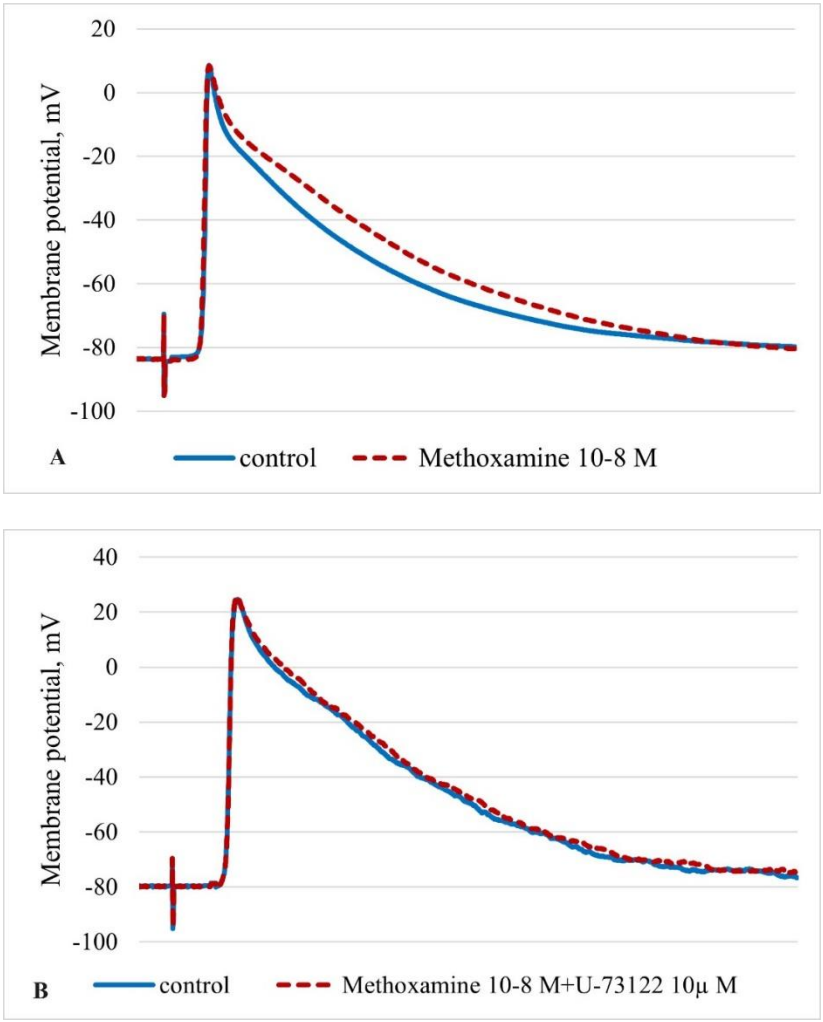

Fig.5. Original recordings of action potentials (AP) of working cardiomyocytes in the right atrium of 100-day-old rats under imposed rhythm upon stimulation of α1-adrenoceptors with methoxamine 10^{-8} M (A) and in the presence of PLC blockade with U-73122 (10 μM) (B)

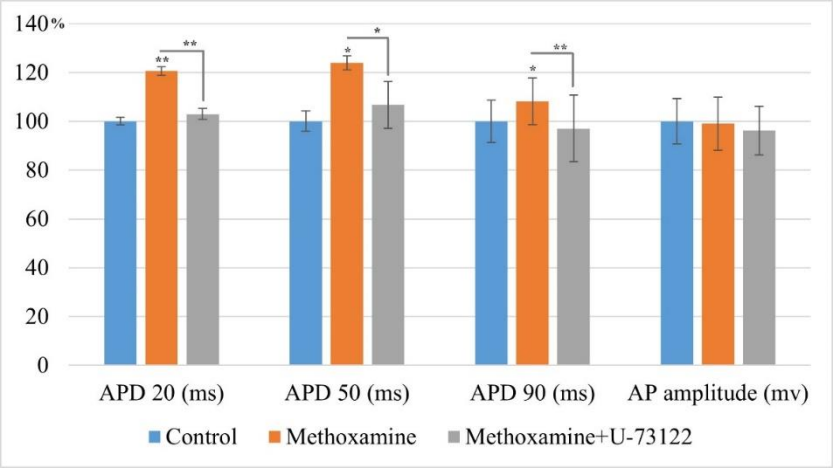

Fig. 6. Percentage effects of methoxamine (10^{-8} M) (n = 8) and methoxamine (10^{-8} M) + U-73122 (10 μM) (n = 7) on amplitude-time parameters in 100-day-old rats under imposed rhythm (note * – $p < 0.05$, ** – $p < 0.01$)

Discussion

In our experiments, we observed the effect of stimulating α1-adrenergic receptors with methoxamine, both alone and in the presence of the selective phospholipase C inhibitor U-73122, on the electrical activity parameters of working cardiomyocytes in rats of different ages under imposed rhythm conditions. The α1-AR agonist methoxamine made changes in the pattern of atrial myocardial electrical activity in 7-, 21- and 100-day-old rats by increasing the repolarization phase of the action potential at levels of APD 20%, APD 50%, and APD 90%. The maximum changes were observed in newborn rats, and the minimum were noted in adult rats. However, there were no changes in the membrane potential, amplitude of the action potential, or duration of the depolarization phase.

The electrical activity of the myocardium is manifested in the form of action potentials, reflecting the activation (and inactivation) of the depolarizing (Na^+, Ca^{2+}) and repolarizing (K^+) current channels (Nerbonne & Kass, 2005). Furthermore, U-73122 is an aminosteroid reported to act as a specific inhibitor of phospholipase C, and has become an important tool in establishing the link between phospholipase C activation and cellular Ca^{2+} signalling (Mogami *et al.*, 1997). In the presence of U-73122, methoxamine treatment in rats of different ages did not change the membrane potential, amplitude of the action potential, duration of the depolarization phase, or the duration of the action potential at APD 20%, APD 50%, or APD 90% of the repolarization phase compared to the control. Methoxamine in the presence of U-73122 markedly blocked all effects of α1-adrenoreceptor stimulation on the parameters of the electrical activity of working cardiomyocytes in 7-, 21-, and 100-day-old rats.

Conclusion

Thus, we conclude that stimulation of α1-adrenoreceptors by methoxamine changes the pattern of electrical activity of the right atrial cardiomyocytes under imposed rhythm by activating the PLC signalling cascade. In rats of different ages, methoxamine increased the duration of the repolarization phase of the action potential. However, when methoxamine was used in the presence of U-73122, these effects were markedly blocked. Additionally, the application of methoxamine, both alone and in the presence of U-73122, did not induce changes in membrane potential, amplitude, or duration of the depolarization phase of the action potential. Our study significantly advances the understanding of age-related and molecular aspects of α1-adrenergic receptor signaling in cardio-myocytes, bridging a key gap in developmental cardiac physiology and paving the way for targeted therapies. It high-

lights the need for further research into intracellular pathways affected by α1-adrenergic receptors and PLC across different ages, providing a roadmap for understanding cardiac physiology at a molecular level.

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The authors declare no conflict of interest.

References

BLEASDALE J.E., THAKUR N.R., GREMBAN R.S., BUNDY G.L., FITZPATRICK F.A., SMITH R.J. & BUNTING S. (1990): Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. *J Pharmacol Exp Ther* **255**(2), 756-768.

BOGOYEVITCH M.A., ANDERSSON M.B., GILLESPIE-BROWN J., CLERK A., GLENNON P.E., FULLER S.J. & SUGDEN P.H. (1996): Adrenergic receptor stimulation of the mitogen-activated protein kinase cascade and cardiac hypertrophy. *Biochem J* **314**, 115-121.

CHANDAN N.R., SMRCKA A.V. & PHAN H.T. (2022): Signaling, Physiology, and Targeting of GPCR-Regulated Phospholipase C Enzymes. GPCRs as therapeutic targets. *John Wiley & Sons, Ltd* **1**, 458-520.

COTECCHIA S. (2010): The α1-adrenergic receptors: diversity of signaling networks and regulation. *Journal of receptor and signal transduction research* **30**(6), 410-419.

HEIN P. & MICHEL M.C. (2007): Signal transduction and regulation: are all alpha1-adrenergic receptor subtypes created equal? *Biochem Pharmacol* **73**(8), 1097-1106.

HOLLYWOOD M.A., SERGEANT G.P., THORNBURY K.D. & MCHALE N. G. (2010): The PI-PLC inhibitor U-73122 is a potent inhibitor of the SERCA pump in smooth muscle. *Br J Pharmacol* **160**(6), 1293-1294.

ICHISHIMA K., YAMAMOTO S., IWAMOTO T. & EHARA T. (2010): alpha-Adrenoceptor-mediated depletion of phosphatidylinositol 4, 5-bisphosphate inhibits activation of volume-regulated anion channels in mouse ventricular myocytes. *Br J Pharmacol* **161**(1), 193-206.

KHABIBRAKHMANOV I.I., KUPTSOVA A.M., ZIYATDINOVA N.I., MANSUR N. & ZEFIROV T.L. (2020): Alpha(1)-adrenoceptors activation decreases myocardial contractility in newborn rats. *Journal of Experimental Biology and Agricultural Sciences* **8**(2), S322-S326.

KOHUTOVA A., MÜNZOVA D., PEŠL M. & ROTREKL V. (2023). α1-Adrenoceptor agonist methoxamine inhibits base excision repair via inhibition of apurinic/apyrimidinic endonuclease 1 (APE1). *Acta pharmaceutica (Zagreb, Croatia)* **73**(2), 281-291.

MANSOUR N., ZIYATDINOVA N.I., GALLIEVA A.M., SHAKIROV R.R. & ZEFIROV T.L. (2023): Effect of α1 Adrenoreceptors Stimulation on Electrical Activity of Rat Atria. *Biophysics (Russian Federation)* **68**, 607-611.

MANSOUR N., ZIYATDINOVA N.I. & ZEFIROV T.L. (2023): methoxamine plays a role in the regulation of the electrical activity of newborn rats. *Opera Med Physiol* **10**, 59-64.

MOGAMI H., LLOYD MILLS C. & GALLACHER D.V. (1997): Phospholipase C inhibitor, U73122, releases intracellular Ca2+, potentiates Ins(1,4,5)P3-mediated Ca2+ release and directly activates ion channels in mouse pancreatic acinar cells. *Biochem J* **324**, 645-651.

NERBONNE J.M. & KASS R.S. (2005): Molecular physiology of cardiac repolarization. *Physiological reviews* **85**(4), 1205-1253.

O'CONNELL T.D., JENSEN B.C., BAKER A.J. & SIMPSON P.C. (2013): Cardiac alpha1-adrenergic receptors: novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. *Pharmacological reviews* **66**(1), 308-333.

SMITH R.J., SAM L.M., JUSTEN J.M., BUNDY G.L., BALA G.A. & BLEASDALE J.E. (1990): Receptor-coupled signal transduction in human polymorphonuclear neutrophils: effects of a novel inhibitor of phospholipase C-dependent processes on cell responsiveness. *J Pharmacol Exp Ther* **253**(2), 688-697.

WOODCOCK E.A. (2007): Roles of alpha1A- and alpha1B-adrenoceptors in heart: insights from studies of genetically modified mice. *Clin Exp Pharmacol Physiol* **34**(9), 884-888.

ZEFIROV T. L., KHABIBRAKHMANOV I. I., ZIYATDINOVA N. I. & ZEFIROV A. L. (2016): Peculiar Aspects in Influence of α1-Adrenoceptor Stimulation on Isolated Rat Heart. *Bull Exp Biol Med* **162**(1), 4-6.

ZHANG J., SIMPSON P.C. & JENSEN B.C. (2021): Cardiac α1A-adrenergic receptors: emerging protective roles in cardiovascular diseases. *Am J Physiol Heart Circ Physiol* **320**(2), H725-H733.

ZIYATDINOVA N.I., KUPTSOVA A.M., GALIEVA A.M., ZARIPOVA R.I., SYNGATULLINA M.I. & ZEFIROV T.L. (2019): Isolate rat heart: age features. *Eurasia J Biosci* **13**, 1375-1381.