DOSE-DEPENDENT EFFECTS OF METHOXAMINE ON MYOCARDIAL ELECTRICAL ACTIVITY OF NEWBORN AND ADULT RATS

N. Mansour[*]*, N.I. Ziyatdinova, T.L. Zefirov*

Kazan (Volga Region) Federal University, 18 Kremlyovskaya St., Kazan, Republic of Tatarstan, 420008, Russia.

* Corresponding author: nourm94@mail.ru

Abstract. This study investigates the effects of the α1-adrenergic receptor agonist methoxamine at various concentrations (10^{-9} to 10^{-6} M) on the frequency and characteristics of spontaneous action potentials in right atrial preparations of newborn and adult rats. In newborn rats, methoxamine at all studied concentrations significantly increased the duration of the action potential repolarization phase, while in adult rats, decreasing it. Interestingly, in adult rats, methoxamine at a concentration of (10^{-6} M), exhibited a dual effect, decreasing the duration of the action potential repolarization phase in some samples while increasing it in others. Stimulation of α1-adrenergic receptors by methoxamine in working cardiomyocytes of the right atrium in both age groups led to an increase in the frequency of action potential generation; however, this effect was more pronounced in newborn rats. These findings highlight the age-dependent effects of α1-adrenergic receptor stimulation on cardiac electrical activity, suggesting that the mechanisms underlying these responses may differ significantly between newborn and adult rats.

Keywords: α1-adrenoreceptor, methoxamine, action potential duration, heart, rat.

List of Abbreviations

AR – Adrenergic receptor
GPCRGs – protein-coupled receptors
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
PI_3K – Phosphatidylinositol 3-Kinase

Introduction

Adrenoceptors have played a crucial role in the history of pharmacology. They were essential components of the research that led to the Nobel Prize in Physiology or Medicine in 1988 and 1994, as well as the Nobel Prize in Chemistry in 2012. These prizes highlighted the significant roles that adrenoceptors have played in our understanding of GPCRs function and the evolution of rational drug discovery. The nearly ubiquitous expression of adrenoceptors and their pleiotropic responses have resulted in the development of successful drugs for a myriad of diseases (Michel *et al.*, 2019).

Adrenoceptors are classified into three main types: α_1, α_2, and β, each of which has three subtypes. The three subtypes of α_1-adrenoceptors are α_1A, α_1B, and α_1D, each exhibiting unique tissue distributions and physiological roles. When activated by agonists such as norepinephrine, these receptors initiate a cascade of intracellular signaling pathways involving coupling to G proteins from the Gq/11 family, subsequently activating phospholipase C (PLC). This activation leads to the formation of two products: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), thereby triggering the PLC-IP_3 pathway (Hein & Michel, 2007). The binding of IP_3 to its receptor on the endoplasmic reticulum activates the calcium-sensitive enzyme protein kinase C (PKC), leading to the release of Ca^{2+} into the cytoplasm. Six PKC isoforms have been detected in neonatal rat cardiomyocytes (Disatnik *et al.*, 1994). In adult rats, only three isoforms remain in the myocardium, while the other PKC isoforms disappear with age (Pucéat *et al.*, 1994). PKC is also considered an important regulator of the IKs current channels. Activation of PKC leads to a reduction in the IKs current in both mouse and rat myocytes. PKC influences other potassium currents found in cardiomyocytes. The phosphorylation of the Na/Ca exchanger by protein kinases enhances its activity. Furthermore, PKC interacts with various targets, including β- AR kinases, muscarinic receptors, transcription fac-

tors, genes, and many others (Steinberg, 2012; Gada & Logothetis, 2022).

Methoxamine is a well-known α_1-AR agonist, clinically used as a longer-acting analogue of epinephrine (Kohutova *et al.*, 2023). Stimulation α_1-AR with methoxamine in the isolated heart of adult rats induces gradually developing bradycardia, which progresses over several minutes. In vivo, similar stimulation produces a short-term bradycardia, likely terminated by compensatory mechanisms in the organism (Zefirov *et al.*, 2016). Studies have shown that the negative effects of α_1-AR stimulation on heart chronotropy exhibit age-related features. Specifically, stimulation of α_1-ARs with methoxamine leads to bradycardia in isolated hearts of rats starting from the third week of postnatal ontogenesis, while it does not affect the newborn rats chronotropy. The absence of a chronotropic response in newborn rat pups may be attributed to the underdeveloped adrenergic innervation of the heart at this age, which impacts the density and maturity of the studied receptors (Khabibrakhmanov *et al.*, 2019). In newborn rats, adrenergic regulation of the heart is characterized by immature sympathetic innervation, in contrast to adult rats. The age of 21 days marks the beginning of adrenergic innervation formation in the rat heart. This difference underscores the importance of conducting studies on animals of various ages (Robinson, 1996). Furthermore, the activation of α_1-ARs by methoxamine significantly affects the studied electrophysiological parameters. In newborn rats, methoxamine increased the duration of the repolarization phase of the action potential under imposed rhythms (Mansour *et al.*, 2023), as it did in adult rats (Mansour *et al.*, 2023). The stimulation of α1-adrenergic receptors by methoxamine alters the pattern of electrical activity in right atrial cardiomyocytes under imposed rhythms by activating the phospholipase C (PLC) signaling cascade (Mansour *et al.*, 2024).

Understanding the effects of different concentrations of methoxamine is crucial for elucidating the dose-response relationship and the physiological implications of α1-adrenergic receptor activation. Variations in concentration can lead to distinct effects on cardiac function; for instance, lower concentrations may enhance parameters such as action potential duration and frequency of spontaneous activity, while higher concentrations may produce opposing effects or exhibit a dual response. By investigating a range of concentrations, researchers can identify the thresholds at which these changes occur and better understand the underlying mechanisms of adrenergic regulation in the heart. Moreover, studying the effects of methoxamine across different age groups is essential, as the physiological responses to adrenergic stimulation can vary significantly with development. This age-related variability is particularly important for understanding how cardiac function matures and how it may be affected by pharmacological agents.

Our study aimed to investigate the effects of the α_1-adrenergic receptor agonist methoxamine at various concentrations on the frequency of action potentials and the electrical activity parameters of right atrial cardiomyocytes with preserved sinus nodes and spontaneous activity in 7- and 100-day-old rats.

Materials and Methods

The experimental protocol was approved by the Ethics Committee of Kazan Federal University (Protocol No. 39 of December 22, 2022). In this study, a total of 87 animals were used: 7-day-old (newborn) albino rats (n = 40) and 100-day-old (adult) albino rats (n = 47), utilizing microelectrode technology. The selection of age groups was based on the significant differences in adrenergic regulation of the heart observed between newborn and adult rats. After anesthesia with urethane (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 Tyrode solution containing: 7.54 g/l NaCl; 0.3 g/l KCl; 0.134 g/l $CaCl_2$; 0.06 g/l $MgSO_4$; 0.14g/l NaH_2PO_4; 1.68 g/l $NaHCO_3$; 0.9 g/l of glucose, and saturated with carbogen (95% O_2, 5% CO_2), at pH 7.2 – 7.4 and a temperature of 37 ± 1 ºC. Intracellular action potentials were recorded via glass micro-

electrodes with a resistance of 25–60 MΩ and tip diameter <1 μm which were made on the day of the experiment on a horizontal puller P-1000 ("Sutter Instruments"). After a waiting period of 35–40 minutes for the preparation to adapt, control signals were recorded. Subsequently, the α_1AR agonist methoxamine (Tocris) was dissolved in the working solution at the concentrations (10^{-9-6} M) for data registration. 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 the action potential duration at 20% (APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) of the repolarization phase. Statistical analysis of the obtained results was performed using GraphPad Prism 9. The normal distribution was assessed using the Shapiro-Wilk test. The significance of differences was evaluated using Multiple paired t-tests. Differences were considered statistically significant at $p < 0.05$.

Results

The effect of α_1-AR stimulation on the electrical activity parameters of working cardiomyocytes in 7-day-old rats with preserved sinus nodes and spontaneous activity

The application of methoxamine at a concentration of (10^{-9} M, $n = 10$, Fig. 1) increased the frequency of spontaneous activity by 12.3% ($p < 0.01$). The duration of the repolarization phase of AP at the level of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) increased by 29.8% ($p < 0.01$), 28.6% ($p < 0.01$), and 17% ($p < 0.01$), respectively.

At a concentration of (10^{-8} M, $n = 10$, Fig. 1), methoxamine increased the frequency of spontaneous activity by 42% ($p < 0.001$). The duration of the repolarization phase of AP at the level of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) increased by 43.5% ($p < 0.001$), 40% ($p < 0.001$), and 27% ($p < 0.001$), respectively.

Methoxamine at a concentration of (10^{-7} M, $n = 10$, Fig. 1) increased the frequency of spontaneous activity by 31.2% ($p < 0.05$). The duration of the repolarization phase of AP at the level of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) increased by 42% ($p < 0.01$), 30% ($p < 0.05$), and 26% ($p < 0.05$), respectively.

At a concentration of (10^{-6} M, $n = 10$, Fig. 1), methoxamine increased the frequency of spontaneous activity by 29.9% ($p < 0.01$). The duration of the repolarization phase of AP at the level of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) increased by 41% ($p < 0.01$), 30.6% ($p < 0.01$), and 19.5% ($p < 0.05$), respectively.

In 7-day-old rats, methoxamine at all tested concentrations did not affect the membrane potential, the amplitude of the action potential, or the duration of the depolarization phase.

The effect of α_1-AR stimulation on the electrical activity parameters of working cardiomyocytes in 100-day-old rats with preserved sinus nodes and spontaneous activity

The application of methoxamine at a concentration of (10^{-9} M, $n = 10$, Fig. 2) in 100-day-old rats increased the frequency of spontaneous activity by 6.9% ($p < 0.001$). The action potential duration at APD_{20}, APD_{50}, and APD_{90} of the repolarization phase decreased by 15.1% ($p < 0.05$), 20.5% ($p < 0.05$), and 23.4% ($p < 0.05$), respectively.

At a concentration of (10^{-8} M, $n = 10$, Fig. 2), methoxamine increased the frequency of spontaneous activity by 9.9% ($p < 0.001$). The duration of the repolarization phase of AP at the level of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) decreased by 27.5% ($p < 0.01$), 23.1% ($p < 0.001$), and 16% ($p < 0.01$), respectively.

Methoxamine at a concentration of (10^{-7} M, $n = 10$, Fig. 2) increased the frequency of spontaneous activity by 10.3% ($p < 0.01$). The duration of the repolarization phase of AP at the level of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) decreased by 17.1% ($p < 0.05$), 14.6% ($p < 0.05$), and 14%, respectively.

At a concentration of (10^{-6} M), methoxamine had divergent effects on the electrical activity of the atrial myocardium in 100-day-old rats. In the first group ($n = 9$, Fig. 2) the duration of the repolarization phase of AP at the level of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) decreased by 12.3% ($p < 0.05$), 12.15% ($p < 0.01$), and 10.5% ($p < 0.05$), respectively. Conversely, in the second group

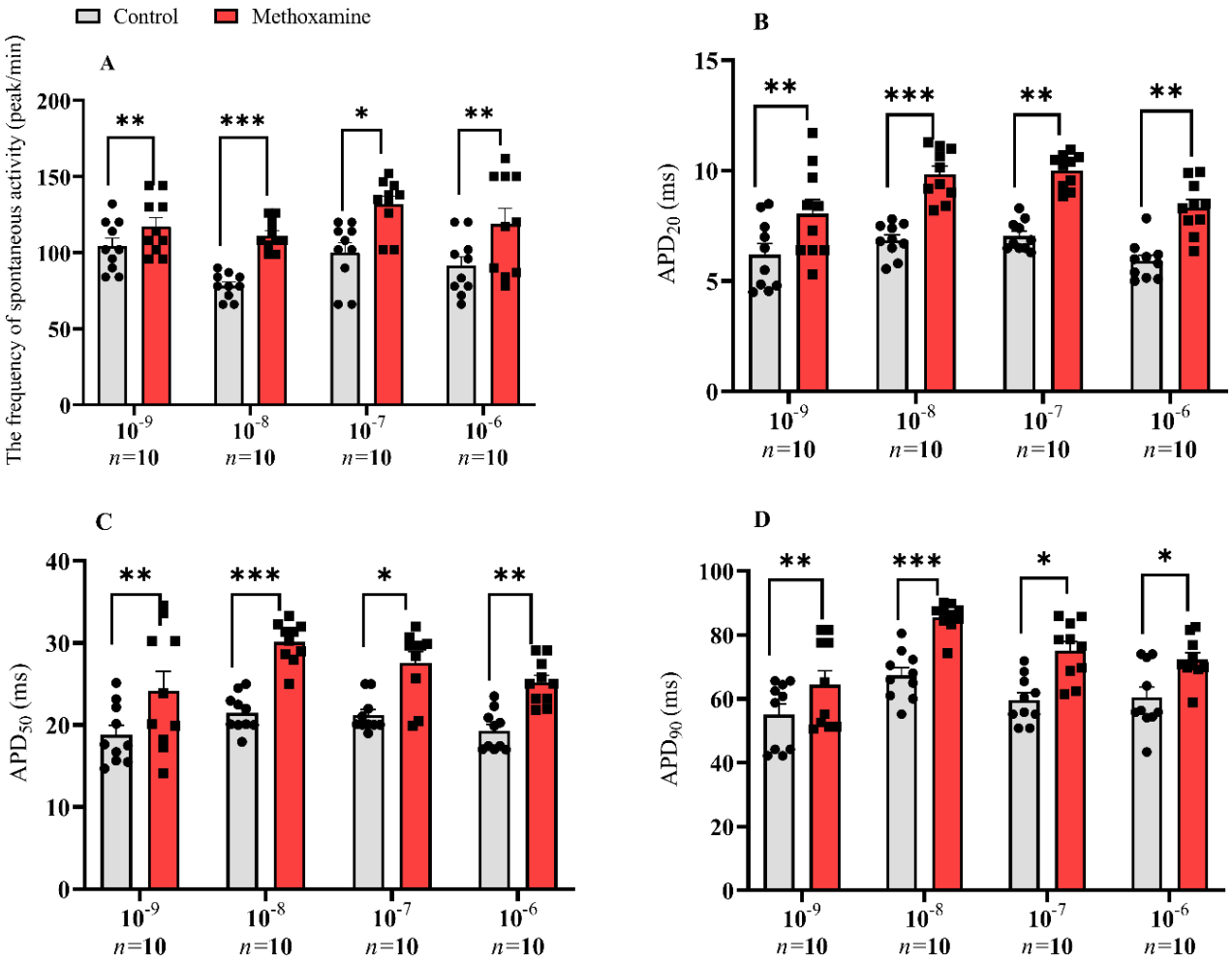

Fig. 1. Effect of methoxamine at different concentrations on the electrical activity parameters of working cardiomyocytes in 7-day-old rats with preserved sinus nodes and spontaneous activity. The parameters assessed include the frequency of spontaneous activity (A), the duration of the repolarization phase of AP at the level of 20%, 50% and 90%: APD_{20} (B), APD_{50} (C), and APD_{90} (D)

(n = 8, Fig. 2) the duration of the repolarization phase of AP at the level of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) increased by 18.4% ($p < 0.05$), 21.6% ($p < 0.01$), and 13.5% ($p < 0.01$), respectively. Additionally, methoxamine increased the frequency of spontaneous activity in both groups by17.13% ($p < 0.01$; n = 9) and 13.7% ($p < 0.001$; n = 8), respectively.

In 100-day-old rats, methoxamine at all tested concentrations did not affect the membrane potential, the amplitude of the action potential, or the duration of the depolarization phase.

Age-dependent differences in electrical activity parameters of working cardiomyocytes in newborn and adult rats upon α_1-AR stimulation

In our study, we observed significant differences in electrical activity parameters between newborn (7-day-old) (n = 10) and adult (100-day-old) (n = 10) albino rats upon α_1-AR stimulation using methoxamine, with preserved sinus nodes and spontaneous activity.

At a concentration of 10^{-8} M, methoxamine increased the duration of the repolarization phase of AP at the levels of 20% (APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) in newborn rats

(Fig. 1), while it decreased it in adult rats (Fig. 2). Additionally, methoxamine significantly increased the frequency of spontaneous activity in both age groups. Importantly, methoxamine did not affect the membrane potential, the amplitude of the action potential, or the duration of the depolarization phase in either age group compared to the control group.

When comparing newborn and adult rats (Fig. 3), significant developmental differences in the cardiac response to α_1-AR stimulation were observed. Methoxamine significantly increased the frequency of spontaneous activity in both age groups, with a 30% greater effect in newborn rats compared to adult rats ($p < 0.001$). Furthermore, the duration of the repolarization phase of the AP at the levels of 20% (APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) was significantly longer in newborn rats than in adult rats, by 70% ($p < 0.001$), 60% ($p < 0.001$), and 40% ($p < 0.01$), respectively.

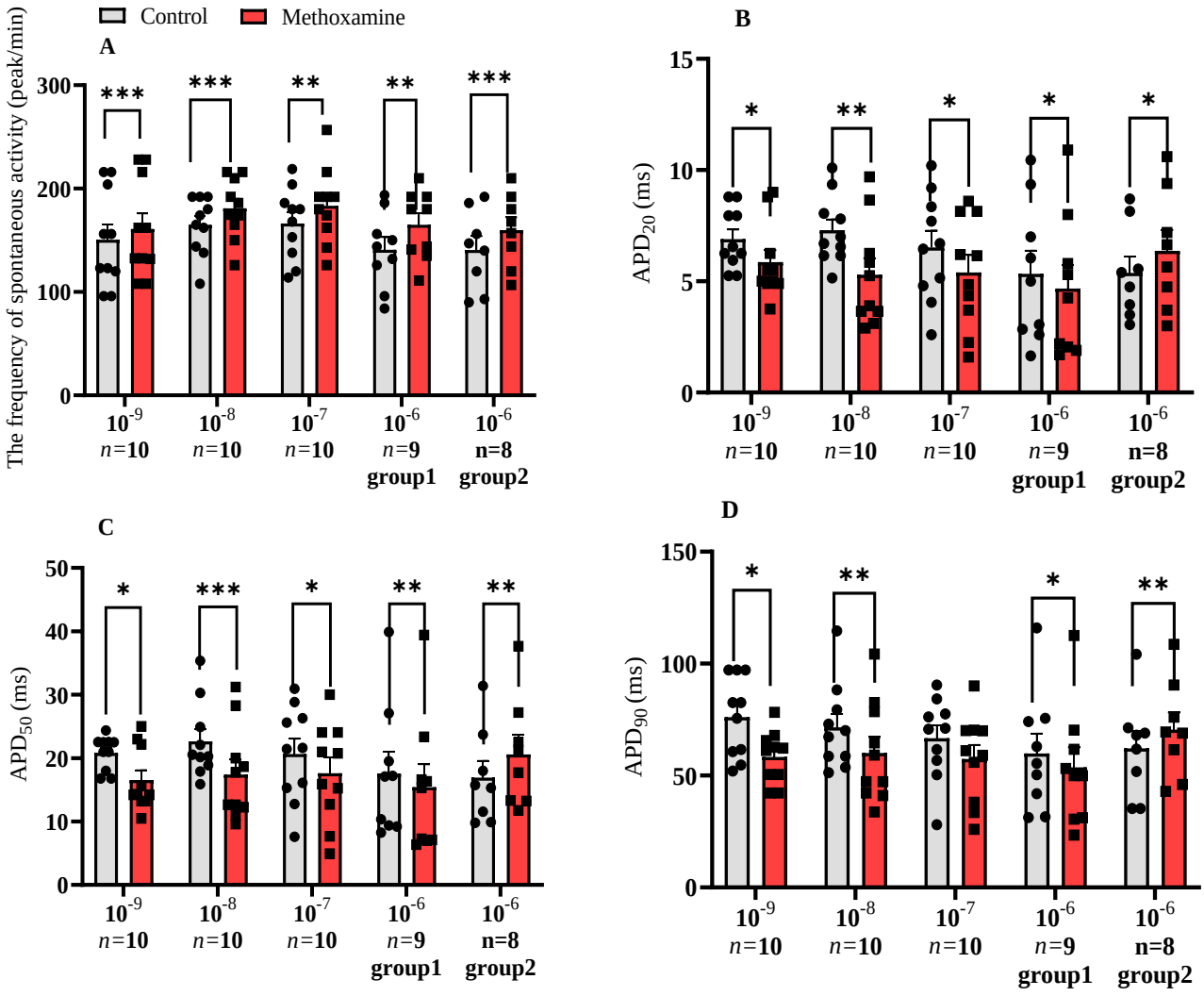

Fig. 2. Effect of methoxamine at different concentrations on the electrical activity parameters of working cardiomyocytes in 100-day-old rats with preserved sinus nodes and spontaneous activity. The parameters assessed include the frequency of spontaneous activity (A), the duration of the repolarization phase of AP at the level of 20%, 50% and 90%: APD_{20} (B), APD_{50} (C), and APD_{90} (D)

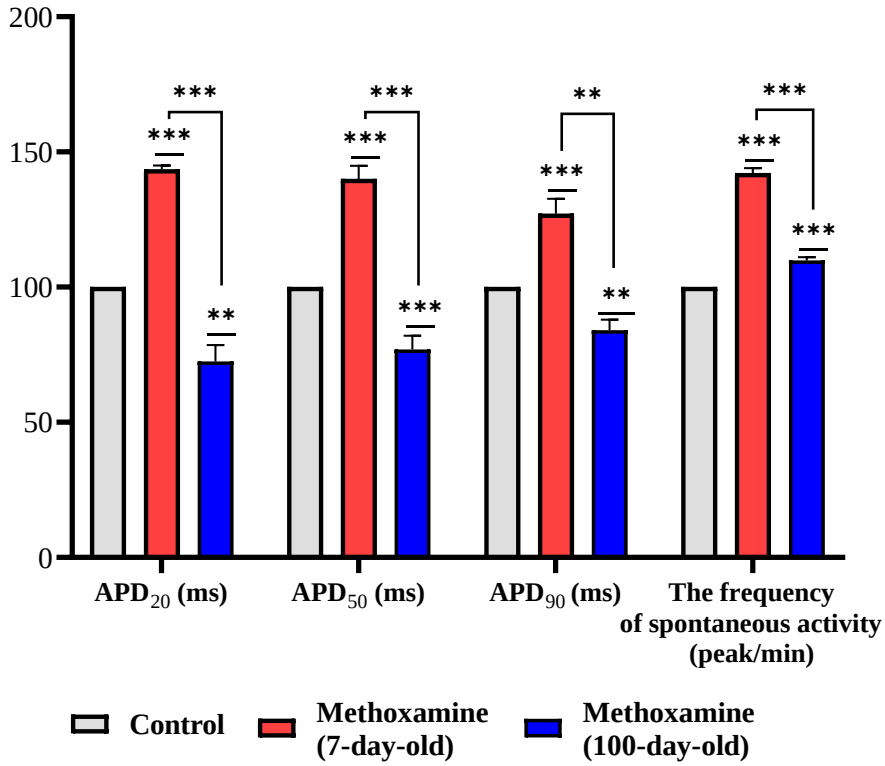

Fig. 3. Comparison of the percentage effects of methoxamine (10^{-8} M) on electrical activity parameters of working cardiomyocytes in 7-day-old (n = 10) and 100-day-old (n = 10) rats

Discussion

In this study, we investigated the effects of the α_1-adrenergic receptor agonist methoxamine at various concentrations on the frequency of action potentials and the electrical activity parameters of right atrial cardiomyocytes with preserved sinus nodes and spontaneous activity in 7-day-old (newborn) and 100-day-old (adult) rats. Our results demonstrated an age-related effect of methoxamine on the repolarisation duration of working cardiomyocytes and the frequency of AP generation in both age groups.

Methoxamine at concentrations (from 10^{-7} to 10^{-9} M) in 100-day-old rats reduced the duration of the action potential at levels of 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) of the repolarization phase. Conversely, at a higher concentration (10^{-6} M) a dual effect was observed. Methoxamine also caused a dose-dependent increase in the frequency of action potential generation in adult rats. In newborn rats, methoxamine at concentrations (from 10^{-6} to 10^{-9} M) led to an increase in the frequency of AP generation, with the maximum effect observed at a concentration of 10^{-8} M. Additionally, methoxamine increased the duration of AP at (APD_{20}), (APD_{50}) and (APD_{90}). The values of action potential amplitude and membrane potential did not change under the effect of methoxamine in either age group. These changes may be attributed to the immature sympathetic innervation in the hearts of newborn rats, in contrast to the more developed sympathetic regulation in adult rats (Robinson, 1996), highlighting the importance of adrenergic regulation during development.

In adult rats, methoxamine at the highest concentrations decreased the repolarization duration in some samples while increasing it in others. Recent studies have indicated that methoxamine exerts positive inotropic effects in the atrium, similar to noradrenaline and phenylephrine; however, at high concentrations, it

may decrease contractile frequency (Zhang *et al.*, 2018). This dual effect could be due to the direct influence of methoxamine on ion channels involved in APD, such as L-type Ca^{+2} channels or K^+ channels.

Comparing newborn and adult rats revealed significant developmental differences in the cardiac response to α_1-AR stimulation. Specifically, methoxamine, an α_1-AR agonist, significantly increased the frequency of spontaneous activity in both age groups; however, the effect was markedly greater in newborn rats. Despite the immature sympathetic innervation in newborn rats, the density and functionality of α_1-ARs may be sufficient to elicit strong responses. Additionally, the physiological adaptations of the immature heart, such as a longer duration of the repolarization phase and altered ionic environments, may enhance reactivity to adrenergic agonists. Furthermore, the duration of the repolarization phase of AP was significantly longer in newborn rats compared to adult rats. These results indicate that the repolarization dynamics in newborn rats are distinct from those in adults, potentially reflecting differences in ion channel expression and function. The observed differences in cardiac responses to methoxamine highlight the importance of age-related changes in cardiac physiology. This maturation process may involve alterations in the expression and activity of key ion channels, such as K^+ channels, which play a critical role in repolarization.

While our findings provide valuable insights into the developmental differences in cardiac electrical activity, there are several limitations to consider. Although the sample size is sufficient for statistical analysis, it may not fully capture the variability within each age group. Additionally, further studies are needed to explore the underlying mechanisms driving these age-dependent differences, including the roles of specific ion channels and intracellular signaling pathways. Furthermore, investigating the effects of various pharmacological agents on both newborn and adult cardiomyocytes could enhance our understanding of age-related differences in cardiac responsiveness.

Conclusion

In conclusion, stimulation of α_1-AR by methoxamine changes the pattern of electrical activity of the right atrial cardiomyocytes with preserved sinus nodes and spontaneous activity. Methoxamine significantly increased the frequency of spontaneous activity in both age groups; however, this effect was more pronounced in newborn rats. In newborn rats, methoxamine increased the duration of the repolarization phase of the AP in a concentration-dependent manner. In contrast, the effects in adult rats were more complex; at lower concentrations, APD decreased, while at higher concentrations, a dual effect was observed.

The observed differences in the effects of methoxamine on APD in the two age groups may be attributed to developmental changes in ion channel expression or signaling pathways. Further research is needed to elucidate the underlying mechanisms and to explore the potential implications for cardiac physiology and pathophysiology.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

DISATNIK M.H., BURAGGI G. & MOCHLY-ROSEN D. (1994): Localization of protein kinase C isozymes in cardiac myocytes. *Exp Cell Res* **210**, 287–297.

GADA K.D. & LOGOTHETIS D.E. (2022): PKC regulation of ion channels: The involvement of PIP2. *J Biol Chem* **298**, 102035.

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

KHABIBRAKHMANOV I.I., ZIYATDINOVA N.I., KHISAMIEVA L.I., KRULOVA A.V. & ZEFIROV T.L. (2019): Age-Related Features Influence of Alpha (1) – Adrenoceptor Stimulation on Isolated Rat Heart. *Biosc. Biotech Res Comm Special* **12**(5), 351–354.

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.

MANSOUR N., ZIYATDINOVA N.I. & ZEFIROV T.L. (2024): Phospholipase c inhibitor prevents the effects of methoxamine on the action potential of cardiomyocytes in rats of different ages. *Opera Med Physiol* **11**, 49–57.

MICHEL M.C., BOND R.A. & SUMMERS R.J. (2019): Adrenoceptors-New roles for old players. *Br J Pharmacol* **176**(14), 2339–2342.

PUCÉAT M., HILAL-DANDANO R., STRULOVICIN B., BRUNTON L.L. & BROWN J.H. (1994): Differential regulation of protein kinase C isoforms in isolated neonatal and adult rat cardiomyocytes. *J Biol Chem* **269**, 16938–16944.

ROBINSON R.B. (1996): Autonomic receptor-effector coupling during postnatal development. *Cardiovasc Res* **31**, 68–76.

STEINBERG S.F. (2012): Cardiac actions of protein kinase C isoforms. *Physiology (Bethesda)* **27**, 130–139.

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,** 4–6.

ZHANG S., TAKAHASHI R., YAMASHITA N., TERAOKA H. & KITAZAWA T. (2018): Alpha1B-adrenoceptor-mediated positive inotropic and positive chronotropic actions in the mouse atrium. *Eur J Pharmacol* **839**, 82–88.