METHOXAMINE PLAYS A ROLE IN THE REGULATION OF THE ELECTRICAL ACTIVITY OF NEWBORN RATS

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Abstract. Various studies have led to our present knowledge of α1-adrenoreceptors and their role in the regulation of cardiovascular physiology. Our study aimed to study the effect of the α1-adrenergic receptor agonist methoxamine on the frequency of spontaneous activity and electrical activity parameters of the atrial myocardium with a preserved sinus node and an imposed rhythm in newborn rats. In working cardiomyocytes of newborn rats with a preserved sinus node, methoxamine caused an increase in the frequency of spontaneous activity. α1-adrenergic receptor agonist methoxamine increased the duration of the repolarization phase of the action potential in both the imposed and the own rhythm.

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

List of Abbreviations

AR – Adrenergic receptor
APD – action potential duration
PLC – Phospholipase C
PIP2 – phosphatidylinositol-4,5-bisphosphate
IP3 – inositol triphosphate
DAG – diacylglycerol
PKC – Protein Kinase C

Introduction

The action of the catecholamines adrenaline and noradrenaline is due to the activation of adrenergic receptors (AR). Noradrenaline is released from postganglionic noradrenergic nerve endings, and both noradrenaline and adrenaline are secreted from the adrenal medulla. Catecholamines are responsible for the control of essential functions such as cardiovascular, respiratory and neuronal functions, digestion, energy metabolism and endocrine function. Thus, the adrenoceptors through which it acts are targets of great therapeutic interest in treating diseases (Zhang *et al.*, 2021). Various studies of adrenoreceptors have led to better knowledge and facilitated their classification. In 1937, Cannon and Rosenblueth (Cannon & Rosenblueth, 1937) proposed a hypothesis based on the presence of two neurotransmitters or endogenous mediators in the body to explain the various reactions (both excitatory and inhibitory) that are observed after the activation of adrenergic receptors. However, the first attempts to classify adrenoreceptors into different groups were made only in 1948. Ahlquist was the first to use synthetic amines, which had a close structural relation with adrenaline, and in his studies, he aimed to evaluate their potency in different organs (Ahlquist, 1948). He found that a group of these amines shared many properties to control several functions in the organism, which are mediated by a common adrenoreceptor, which he called alpha (α). He also found that the range of potency of these same amines was very different when mediating another group of responses in the organism. Ahlquist suggested that this difference in the behaviour of the amines was because of different receptors involved in the former reactions. To distinguish it from the α receptor, he called this second adrenoreceptor the beta receptor (β). Ahlquist was the first to introduce a pharmacological classification of adrenoreceptors. α-adrenoreceptors mediate most excitatory functions like vasoconstriction, uterine muscle contraction, urethral contraction and one inhibitory function (bowel relaxation). However, β-adrenoreceptors mediate most inhibitory function like vasodilation, uterine muscle relaxation, bronchodilation and one excitatory function (cardiac function) (Lands *et al.*, 1967). In 1974, as a result of his in vitro experiments, Langer suggested that the postsynaptic α-adrenoreceptor mediating responses in the effector organ should be called

the α1-adrenoreceptor and that the presynaptic α-adrenoreceptor regulating noradrenaline release should be called the α2-adrenoreceptor (Langer, 1974). Later it was found that some α-adrenoreceptors in postsynaptic locations exhibited a pharmacological affinity similar to that of presynaptic α2-adrenoreceptors, and therefore it was confirmed presence of both pre- and postsynaptic α2-adrenoreceptors. These studies led Betherseln and Pettinger to propose a functional classification of α-adrenoreceptors according to the function mediated by each receptor outside its anatomical site (Berthelsen & Pettinger, 1977). With the development of new high-selectivity drugs for different types of α-adrenoreceptors, a more accurate differentiation of these receptors was established, depending on the pharmacological affinity of each. This differentiation of α-adrenoreceptors was independent of their location relative to the nerve synapsis and the function mediated by their activation. Consequently, the α-adrenoreceptor activated by methoxamine or phenylephrine and competitively inhibited by low concentrations of prazosin and the compound WB-4101 was termed an α1-adrenoreceptor. In contrast, those receptors that responded to activation by the compounds B-HT 920 and UK-14, 304 and which were competitively inhibited by low concentrations of yohimbine, rauwolscine or idazoxan were classified as α2-adrenoreceptors (Civantos & Aleixandre, 2001).

In the late 1980s, α1-ARs were subdivided into α1A- and α1B-AR subtypes based on experimental data of two-site competitive binding curves to the antagonists WB4101 and phentolamine in the rat brain. α1A-AR subtype has a higher affinity for these ligands by 10-100-fold than the α1B-AR subtype (Morrow & Creese, 1986). Several years later, another receptor with a high affinity for WB4101 was cloned and designated the α1D-AR (Perez *et al.,* 1991). All three α1-adrenoreceptor subtypes (α1A-, α1B-, α1D-) are G-protein-coupled receptor (GPCR). The intracellular signal transduction pathways linked to α1-adrenoreceptors are becoming clearer. The α1-adrenoreceptor activates the Gq/11 and leading to the dissociation of the α and βγ subunits. The Gαq/11-subunit stimulates phospholipase C (PLC-β), leading to hydrolysis of the cell membrane phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol triphosphate (IP3), which releases intracellular Ca^{2+} and diacylglycerol (DAG), which stimulates protein kinase C. α1-adrenoreceptors activation in cardiomyocytes increase intracellular calcium concentration by inducing Ca^{2+} release from the endoplasmic reticulum (Hieble *et al.,* 1995; Marshall *et al.,* 1999). Intravenous administration of human α1-AR agonists, particularly methoxamine, has been shown to increase cardiac contractility (Curiel *et al.,* 1989). Recent studies in rats have shown that stimulation of α1-adrenoreceptors by methoxamine reduces the contraction rate of the isolated adult rat heart. The severity of the effect depends on the concentration of the agonist. Intravenous administration of methoxamine also leads to cardiac bradycardia in the whole organism (Zefirov *et al.,* 2016). The adrenergic regulation in newborn rats' heart has an immature sympathetic innervation, for this, the studies on animals of this age are of particular interest (Ziyatdinova *et al.,* 2019; Khabibrakhmanov *et al.,* 2020).

Our study aimed to investigate the role of the α1-adrenoreceptor agonist methoxamine in the regulation of atrial myocardial electrical activity in newborn rats.

Materials and Methods

The study was performed on white albino rats. Rats were housed in cages with free access to water and food. The experimental protocol was approved by the Ethics Committee of Kazan Federal University by ethical principles. All measures were taken to minimize the number of animals used in the experiments. The experiments were carried out on newborn (7-day-old) rats (n = 14). After anaesthesia with urethane (25%), the chest was opened then the heart was excised and transferred to a Petri dish. After that, an isolated right atrial myocardium with preserved sinus node and spontaneous activity was prepared. During the experiment, the right atrial preparation was constantly perfused with a solution containing (in mM): 133.47 NaCl,

4.69 KCl, 1.35 $NaH_2PO_4 \times 2H_2O$, 16.31 $NaHCO_3$, 1.18$MgSO_4 \times 7H_2O$, 2.5 $CaCl_2 \times 2H_2O$, 7.77 glucose and saturated with carbogen (95% O_2, 5% CO_2), the pH was maintained at 7.2-7.4 at (37 ± 1ºC). Further, the effects of alpha-1-adrenoreceptor stimulation were studied on atria with their own and imposed rhythm. In experiments with an imposed rhythm, atria were stimulated with platinum electrodes (3 Hz). Intracellular action potential was recorded via glass microelectrodes with a resistance of 25-60 MΩ. The signals were digitized with an E14-140 converter (L-Card) and recorded using Elph 3.0 software. Data were processed using Excel. Registration of the membrane potential, the action potential, the duration of depolarization, the amplitude of action potential, and the duration of the action potential, was determined at the level of 20% (APD 20%), 50% (APD 50%) and 90% (APD 90%) of the repolarization phase. The alpha-1-adrenoreceptor agonist methoxamine (Tocris) was used at a concentration of 10^{-8} M based on previously conducted experiments. It was dissolved in the work solution and applied after control recording. The results of the experiments are expressed as mean ± standard error of the mean. Statistical significance was evaluated using Student's test to compare the two groups. Values at * $p < 0.05$ were considered significant.

Results

Effect of methoxamine on the electrical activity parameters of working cardiomyocytes in newborn rats with preserved sinus node and spontaneous activity.

Methoxamine at a concentration of 10^{-8} M (Fig. 1.) increased the duration of action potential at APD 20%, APD 50% and APD 90% by 44% ($p < 0.05$), 40% ($p < 0.05$), and 27% ($p < 0.05$) respectively, while the duration of depolarisation phase did not change. The values of action potential amplitude and membrane potential also did not change. Methoxamine at a concentration of 10^{-8} M in newborn rats increased the frequency of action potential generation by 42.3% ($p < 0.05$).

In order to exception the effect of methoxamine on frequency component of working cardiomyocytes, in the next series of experiments we studied the effect of methoxamine on the parameters of the electrical activity with imposed rhythm in newborn rats.

The application of methoxamine at a concentration of 10^{-8} M (Fig. 2.) in 7-day-old animals did not change the membrane potential and action potential amplitude. The duration of the action potential at APD 20%, APD 50% and APD 90% increased by 57% ($p < 0.05$), 54% ($p < 0.05$) and 41% ($p < 0.05$), respectively.

Discussion

In our experiments, we observed the effect of methoxamine on the repolarisation duration, and on the time between beaks of working cardiomyocytes. With self- and imposed rhythm, methoxamine made changes in the pattern of atrial myocardial electrical activity in newborn rats by increasing the repolarisation phase of the action potential. The α1-AR agonist had been shown to affect repolarisation duration at the level of APD 20%, APD 50%, and APD 90% in 7-day-old rats. The maximum changes were observed at the APD 20% level and the minimum at the APD 90% level. However, there were no changes in membrane potential, action potential amplitude and duration of the depolarisation phase.

The electrical activity of the myocardium manifests itself in the form of action potentials, reflecting the activation (and inactivation) of the depolarizing (Na^+, Ca^{2+}) and repolarizing (K^+) current channels. Several types of myocardial K^+ channels contribute to regional differences in action potential waveforms and in the generation of normal heart rhythms (Nerbonne & Kass, 2005). The increase in the frequency of action potential generation may be related to the effect of methoxamine on the time between peaks. The Na^+ current channels accomplish this by increasing excitability and promoting post-repolarization refractoriness (PRR). On the other hand, the prolong of action potential duration (APD) is principally due to the decrease in IK1 (inwardly rectifying potassium current) and IK–ACh (acetylcholine-activated potassium current) and IKur (ultra-rapid delayed rectifier potassium current) (Antzelevitch & Burashnikov, 2010; Grandi, 2017).

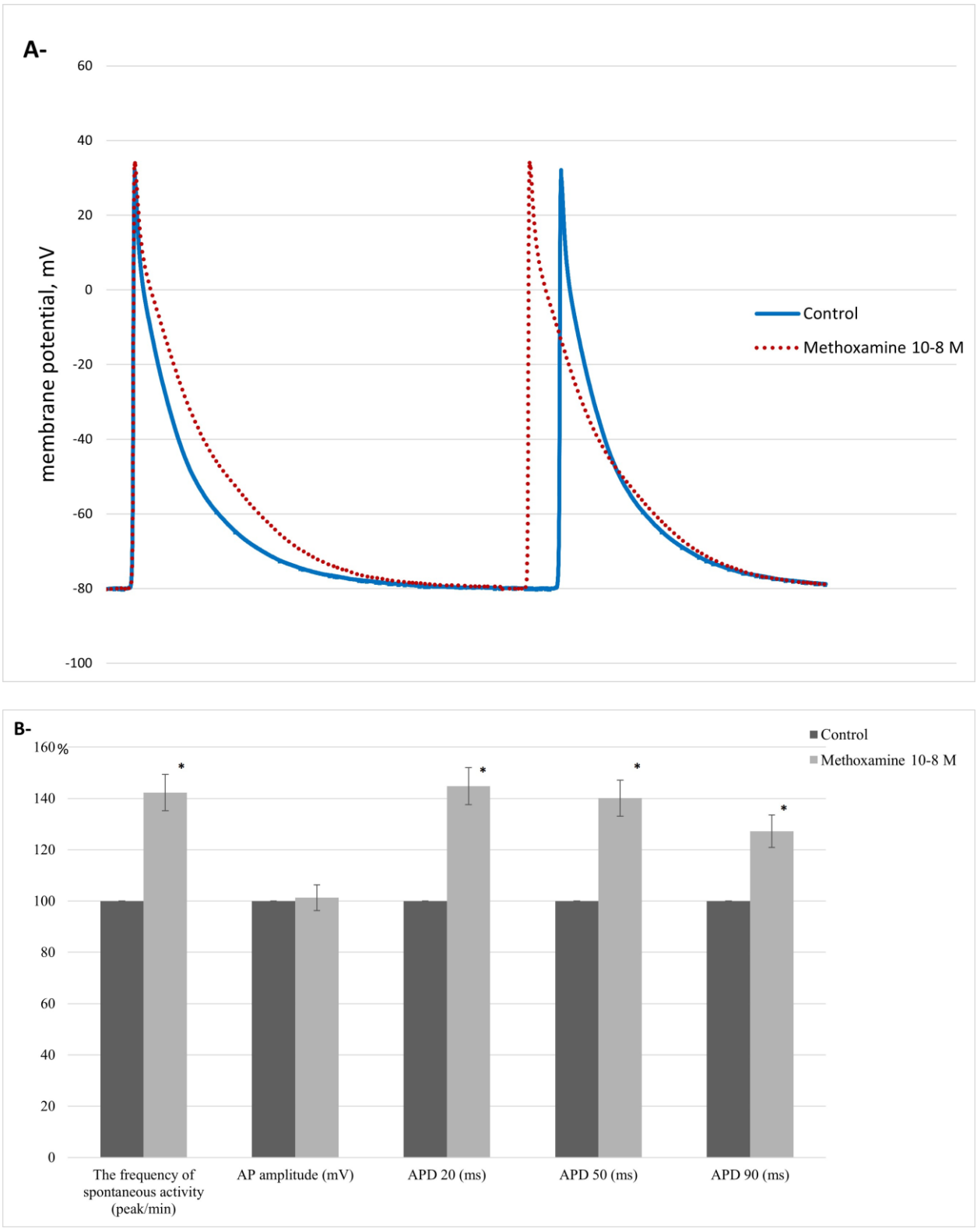

Fig. 1. Effect of methoxamine (10^{-8} M) on amplitude-time parameters in 7-day-old rats with preserved sinus node and spontaneous activity (A – original recording; B – percentage effect) (*Note*: * – $p < 0.05$) (n = 7)

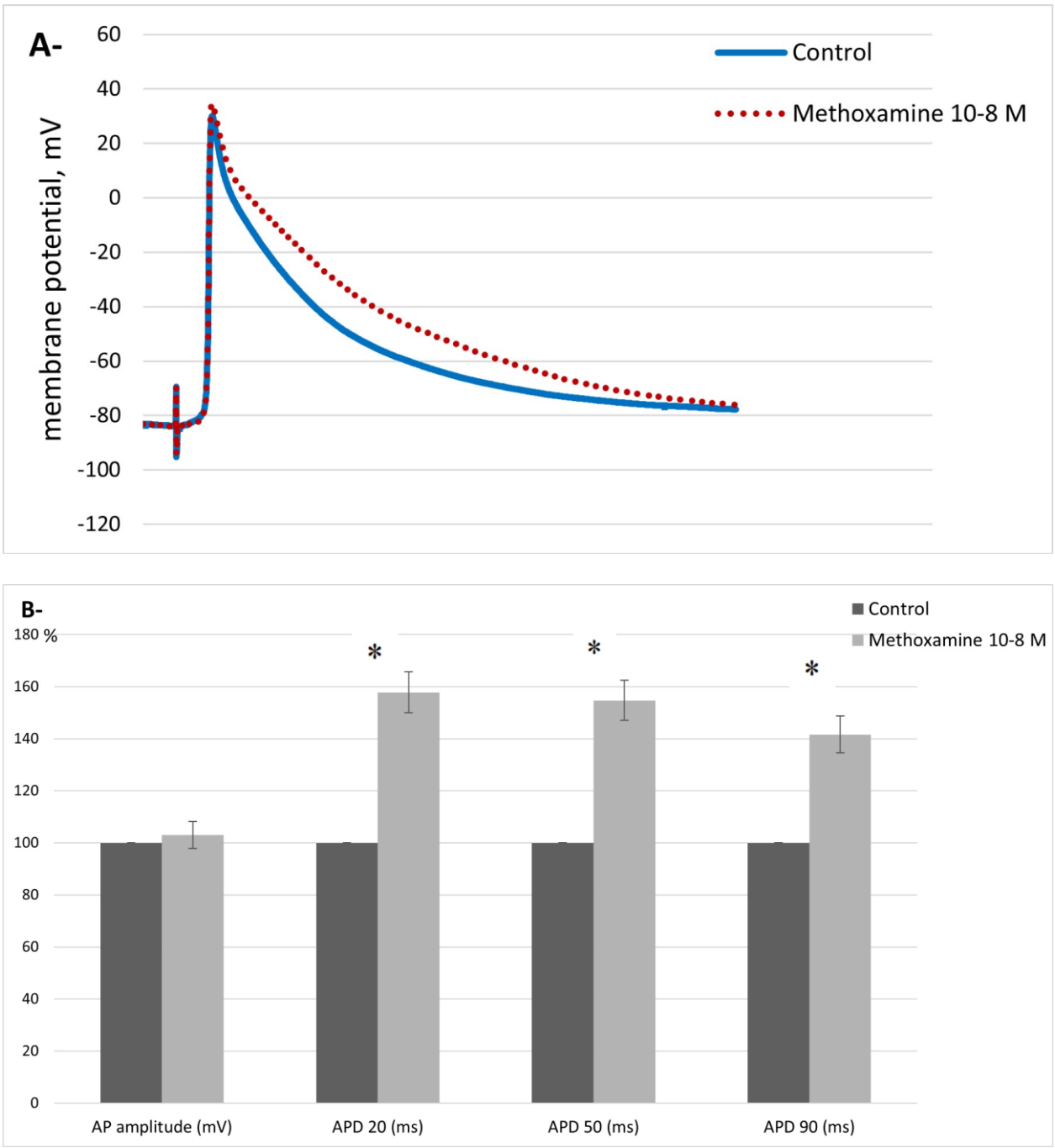

Fig. 2. Effect of methoxamine (10^{-8} M) on amplitude-time parameters in 7-day-old rats with imposed rhythm (A – original recording; B – percentage effect) (*Note:* * – $p < 0.05$) (n = 7)

Conclusion

Thus, it is concluded that stimulation of α1-adrenoreceptors in rats' right atrial working cardiomyocytes with self- and imposed rhythm does not cause changes in membrane potential, amplitude and duration of action potential depolarisation phase. At the same time, methoxamine affected the duration of action potential repolarization in newborn rats. It should be noted that in experiments with both self- and imposed rhythm, methoxamine led to an increase in the duration of the repolarisation phase of the action potential. We have shown that the effect of methoxamine on the electrical activity of newborn rats has significant features compared with adult rats.

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