DOI 10.1007/s10517-016-3500-5

Effect of $I_{Ca,L}$ Blockade on Adrenergic Stimulation in Developing Heart

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 161, No. 6, pp. 696-699, June, 2016
Original article submitted August 23, 2015

The effect of verapamil-induced blockade of L-type calcium ionic currents ($I_{Ca,L}$) on the action of non-selective adrenergic cardiac stimulation by norepinephrine was examined during different periods of early postnatal ontogeny. In 1-week-old rats, intravenous norepinephrine induced a short-term tachycardia both with and without preliminary injected verapamil. In 3-week-old rats, norepinephrine alone produced no chronotropic effect; in contrast, it induced a biphasic tachycardia in verapamil-treated rats. In 6- and 20-month-old rats, norepinephrine induced a short-term tachycardia, which could be prevented by verapamil. The age-related peculiarities of chronotropic action of non-selective adrenergic stimulation are indicative of the role of L-type calcium ionic channels in the development of sympathetic control over the heart.

Key Words: *adrenoreceptors; L-type Ca^{2+} channels; chronotropy; ontogeny; heart*

The heart is a sophisticated automatic device, whose rhythmical performance is controlled by the neurohumoral mechanisms capable to adapt the heart rate and cardiac output according to body needs and variable environmental conditions [9].

Important cardiac control pathways include the membrane adrenoreceptors (AR) that had been subdivided into α and β subtypes by R. P. Ahlquist in 1948 [7]. Further studies revealed the presence of a broad spectrum of various receptors in the heart and other organs involved in the control of numerous vital physiological functions including smooth muscle contraction, maintenance of vascular tone, BP, inotropy, chronotropy, and glucose metabolism in the liver. In addition, α-AR can play the adaptive and protective functions in the heart [13].

α-AR are subdivided into α_1 and α_2 classes, each of which is further divided into 3 subtypes: α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}. In their turn, β-AR are subdivided into β_1 and β_2 classes. The proportion of β-AR and α-AR in the heart is 90 and 10%, respectively. However, the rat heart is an exception with a 10-fold higher proportion of α-AR [11,12]. In general, AR represent the largest receptor group in an organism.

Similar to the cells in other organs, the cardiomyocytes are controlled by the membrane-bound proteins known as G protein-coupled receptors (GPCR). β-AR are the key GPCR expressed in cardiomyocytes and playing an important role in the heart as the regulate the inotropic and chronotropic sympathetic influences.

β-AR are coupled to Gs protein. Stimulation of these receptors activates classical signaling cascade engaging adenylate cyclase, cAMP, and protein kinase A and PKA-dependent protein phosphorylation [10]. Activated α_1-AR bind to Gq/11 protein and trigger signaling cascade leading to generation of phospholipase C, elevation of intracellular diacylglycerol and inositol triphosphate, intracellular calcium mobilization, and activation of protein kinase C [8]. The role of activated α_2-AR consists in inhibition of adenylate cyclase which down-regulates intracellular cAMP.

The transmembrane transport of Ca^{2+} ions in the myocardium is performed via voltage-dependent L-

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type calcium channels [6]. In the heart, these channels are also important for shaping the electrical and mechanical behavior of the myocardium, where they are involved in the regulation of excitation and contraction, hormonal secretion, and gene expression; in addition, they play the key role in determining the length of refractory period of cardiomyocyte action potential [5].

The effects of transmitters of the autonomic nervous system and activity of intracellular signaling cascades can differ at different stages of ontogeny [1-4]. We examined the effect of norepinephrine (NE), a non-selective AR agonist, on heart performance in 1-, 3-, 6-, and 20-week-old rats under normal conditions and during blockade of L-type calcium ionic currents ($I_{Ca,L}$) with verapamil.

MATERIALS AND METHODS

The experiments were carried out on random-bred albino rats aging 1, 3, 6, or 20 weeks (n=52). The rats were anesthetized intraperitoneally with 25% urethane (800 mg/kg body weight). NE (0.01 mg/kg) and/or verapamil (0.1 mg/kg) were injected into the right femoral artery (in combined administration, verapamil was the first injected agent). ECG was recorded in standard lead II via subcutaneous stainless steel needle electrodes connected to an EK 1T-03M electrocardiograph, a C1-83 monitor, and PC. Original software calculated 12 parameters of variational pulsogram. In this study, we analyzed the mean cardiac interval X_m (inverse value of the mean HR).

The data were processed statistically using Microsoft Excel software, Student's t test, and Wilcoxon's test at p<0.05.

RESULTS

In mature rats aging 20 weeks, NE provoked significant although short-term tachycardia. By postinjection minute 3, it decreased X_m from 197±9 to 173±7 msec (p<0.05). Then, no significant changes in X_m were observed. NE administered to verapamil-pretreated rats induced no significant changes in the variational pulsogram parameters. By 15 min of combined use of verapamil and NE, X_m decreased from 185±16 to 171±17 msec (Fig. 1).

Injection of NE to 6-week-old rats decreased X_m from 143±5 to 124±2 msec (p<0.01, Fig. 1). By minute 3 after individual injection of NE, X_m began to restore and attained 145±5 msec by postinjection minute 15. NE applied against the background of verapamil induced no significant changes in cardiac rhythm; X_m tended to decrease (from 151±6 to 141±5 msec; Fig. 1). Other parameters of variational pulsogram

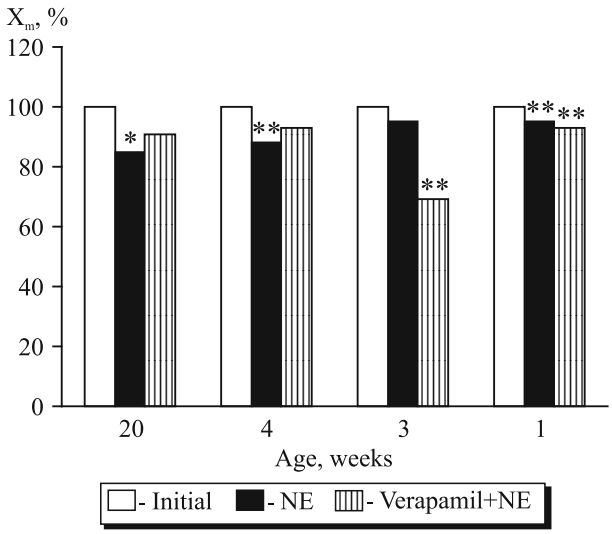

Fig. 1. Effects of NE alone and in combination with verapamil on heart performance at different stages of postnatal ontogeny. Here and in Fig. 2: *p<0.05, **p<0.01 in comparison with initial values (prior to administration of the tested agents) taken for 100%.

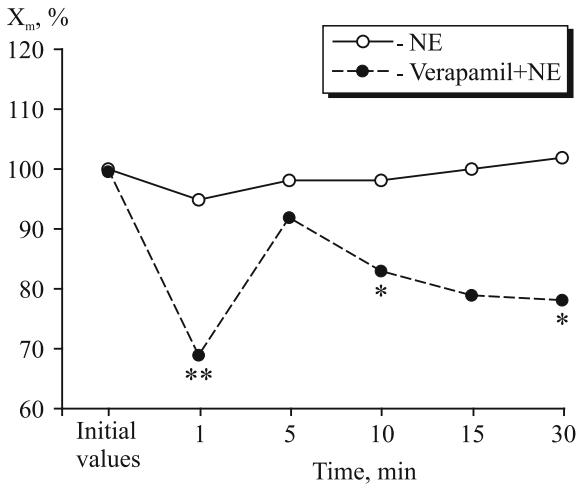

Fig. 2. Effects of norepinephrine alone and in combination with verapamil on the mean cardiac interval in 3-week-old rat pups.

were also unchanged after combined use of verapamil and NE in 6-week-old rats.

Injection of NE to 3-week-old rats produced no significant changes in the examined parameters. X_m decreased from 124±3 to 119±3 msec on postinjection minute 1, and then returned to initial level of 124±6 msec by minute 15 (Fig. 2). Combined administration of both examined agents induced significant biphasic acceleration of the heart rhythm. On minute 1 after NE injection, X_m decreased from 200±11 to 139±13 msec (p<0.01, Fig. 2), thereafter this value began to restore. However, on postinjection minute 10, X_m decreased again to 167±9 msec (p<0.05) and attained the level of 157±11 msec to postinjection minute 30 (p<0.05, Fig. 2).

Injection of NE to 1-week-old rat pups provoked tachycardia. On postinjection minute 1, X_m decreased

from 133.7±0.7 to 127.6±0.5 msec ($p<0.01$, Fig. 1). On postinjection minutes 10 and 15, the values of X_m were 130.4±0.2 msec ($p<0.01$) and 131.6±0.6 msec ($p<0.01$), respectively. In verapamil-injected pups, NE also induced tachycardia. On NE postinjection minute 1, X_m decreased from 126±2 to 118±8 msec ($p<0.01$, Fig. 1). On minute 5, it slightly restored to the level of 121±2 msec ($p<0.05$).

Examination of cardiac chronotropy under combined application of $I_{Ca,L}$ blocker verapamil and a non-selective AR agonist NE revealed significant age-related peculiarities in cardiac responses to AR stimulation. Under these conditions, NE increased the heart rate in rats aging 1, 6, and 20 weeks, but it produced no chronotropic effect in 3-week old rats. $I_{Ca,L}$ blockade prevented NE-induced tachycardia in 6- and 20-week-old rats. In contrast, such blockade could not prevent NE-induced tachycardia in 1-week-old rat pups.

The most interesting data were obtained on 3-week-old rats. In verapamil-pretreated rats, NE induced more severe tachycardia than in verapamil-free animals. In other word, $I_{Ca,L}$ blockade promoted the chronotropic (tachycardiac) effect of NE. It should be noted that NE-induced tachycardia in verapamil-pretreated 3-week-old rats was biphasic (Fig. 2).

Modern literature reports the data on increasing expression of L-type calcium ionic channels in ontogeny [15]. The present data indicate a significant role of $I_{Ca,L}$ in realization of adrenergic regulation in adult and in 6-week-old rats in contrast to the neonatal rat pups. The age-related increase of the density of L-type calcium ionic channels was also demonstrated *in vitro* by employing sympathetic innervation to stimulate the development of $I_{Ca,L}$ [15]. The development and consolidation of cardiac sympathetic innervation in rats begin at the age of 3 weeks and terminate in 6-week-old rats [14], so in this study we examined the rats at these ages. The study revealed essential peculiarities in NE-induced tachycardia in verapamil-pretreated 3-week-old rats, which attest to the functionally importance changes in $I_{Ca,L}$ at this period of ontogeny.

The study was supported by the Russian Foundation for Basic Research (grant No. 15-04-05384).

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