PHYSIOLOGY

Peculiar Effects of Muscarinic M_1, M_2, and M_3 Receptor Blockers on Cardiac Chronotropic Function in Neonatal Rats

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The effects of muscarinic M_1, M_2, and M_3 cholinergic receptor blockade on the regulation of chronotropic function of the heart were studied *in vivo* in 7-day-old rat pups. Intravenous injection of M_2 receptor blocker gallamine produced no changes in cardiac chronotropy. In contrast, M_1 receptor blocker pirenzepine and M_3 receptor blocker 4DAMP provoked bradycardia. These data attest to the involvement of M_1 and especially M_3 cholinergic receptors in the regulation of cardiac chronotropy in rat pups, which confirms the view on pronounced species-specific and age-related peculiarities in the heart control mechanisms.

Key Words: *heart; muscarinic M_1-, M_2-,* and *M_3-cholinoreceptors*; *vagus*; *rat*; *ontogeny*

The parasympathetic branch of the autonomic nervous system realizes its effects via muscarinic cholinoceptors (MCR). In the heart of various mammal species, M_2CR is the predominant fraction [5,8]. Probably, this explains the fact that M_1 receptors received little attention. However, recently the researchers focused on M_1 and M_3 receptors with particular emphasis on the latter [1,2,7,12]. A hypothesis was put forward that interaction of Ach with various MCR subtypes, activation of the second messenger systems, and modulation of the activity of various effectors underlie some features of cardiac control [4,6,9-11]. There are specious-specific peculiarities in the expression of various types and subtypes of the cholinoceptors in mammalian myocardium. However, the role of various subtypes of MCR in physiological, pathophysiological, and pharmacological processes is still unclear. Elucidation of the role of various MCR subtypes in myocardium of animals at different stages of maturation of the sympathetic innervation is an important problem.

Our aim was to study the effect of selective blockade of various subtypes of MCR on heart rate variability in neonatal rats.

MATERIALS AND METHODS

The study was carried out on random-bred 7-day-old albino rat pups (n=22) narcotized intraperitoneally with 25% urethane (1000 mg/kg body weight). The hair in the operative field was sheared off shortly and the skin was treated with iodine and alcohol solutions. M_1CR blocker pirenzepine, M_2CR blocker gallamine, and M_3CR blocker 4DAMP (all reagents were from Sigma) were injected into femoral artery (0.02 mg/kg, bolus injections). During the experiments, ECG was continuously recorded and processed using original software to obtain ECG, BP, and 28 parameters of variation pulsogram [3].

The data were analyzed statistically using Microsoft Excel software. Significance of differences was assessed using Wilcoxon test and Student t test.

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RESULTS

For evaluation of the role of MCR subtypes in vagal control over the heart in neonatal rats, three series of experiments were carried out. In series I, II, and III, the specified parameters of cardiovascular systems were recorded before and during application of M_1CR, M_2CR, and M_3CR blockers, respectively. It should be remembered that neonatal rats have no sympathetic innervation in the heart.

M_1CR blocker pirenzepine gradually increased the mean cardiac interval X_m, by the 15th minute postinjection (Fig. 1). On postinjection second 30, it insignificantly increased from 187.3±5.9 to 191.8±4.9 msec. The insignificant elevation of X_m was observed on minutes 1, 3, and 5 to 192.4±6.5, 197.6±4.5, and 200.4±6.1 msec, respectively. Finally, the difference from the baseline value became significant by minute 15 (p<0.5), when X_m attained the value of 205.1±4.8 msec (Fig. 1). Pirenzepine also changed the parameters of variational pulsogram. The mode amplitude (AMo) significantly decreased from 61.1±2.5 to 53.4±2.0% and 51.1±1.9% on postinjection minutes 1 and 3, respectively (p<0.05); by postinjection minute 15 this parameter decreased to 41.1±6.5% (p<0.01). On postinjection second 30, the variational range dX insignificantly increased from 3.6±0.2 to 3.8±0.1 msec. On the following, dX significantly increased on minutes 1, 3, and 5 to 4.4±0.3 msec (p<0.05), 4.6±0.3 msec (p<0.01), and 4.7±0.4 msec (p<0.01). However, the increase in dX became insignificant on minute 15, when it attained the value of 10.9±6.5 msec.

In contrast to pirenzepine, gallamine produced only insignificant gradual increase in X_m from the baseline value of 172.4±10.7 to 175.7±11.2, 176.4±11.2, 179.5±11.1, 180.3±11.6, and 182.5±12.6 msec on postinjection second 30 and minutes 1, 3, 5, and 15, respectively (Fig. 1). Other parameters of variational pulsogram also changed insignificantly.

The effect of 4DAMP on X_m was not monotonic (Fig. 1). At first, this M_3CR blocker significantly increased X_m from the baseline value of 241.5±6.7 to 332.1±15.6 (p<0.01), 264.4±8.6 msec (p<0.05), and 271.5±9.2 msec (p<0.01) on postinjection second 30 and minutes 1 and 3, respectively. However, X_m decreased to 257.0±13.8 msec on minute 5 (and became insignificantly different from the starting value), but significantly increased again to 266.7±12.9 msec on minute 15 (p<0.05, Fig. 1).

Thus, our experiments revealed the chronotropic response of the heart in neonatal rats to blockade of

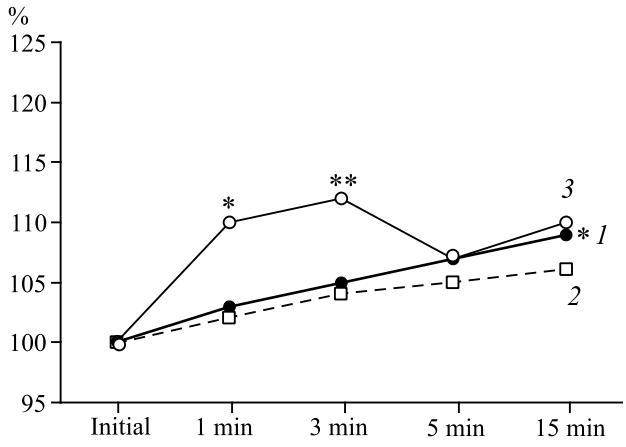

Fig. 1. Dynamics of cardiac interval X_m during selective blockade of various subtypes of M-cholinoreceptors. *1*) pirenzepine; *2*) gallamine; *3*) 4DAMP.

M_1 and M_3 cholinoceptors. The corresponding MCR-blockers significantly decreased the heart rate, the effect of M_3CR blocker being more pronounced. It is noteworthy that electrical stimulation of the vagus nerve also induces bradycardia in rat pups [3]. These findings suggest that the tonic vagal influences on the heart rate are opposite in neonatal and mature rats [2]. The absence of sympathetic innervation in the heart of neonatal rats is probably compensated by additional accelerating chronotropic mechanisms.

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