M_3 Cholinergic Receptors Are Involved in Postnatal Development of Cholinergic Regulation of Cardiac Activity in Rats

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We studied the role of M_3 cholinergic receptors in the regulation of cardiac activity in rats during early postnatal ontogeny *in vivo*. Blockade of M_3 cholinergic receptors in 20-week-old animals increased heart rate and decreased blood pressure. In rats aging 8, 6, and 3 weeks, blockade of M_3 cholinergic receptors had little effect on *R-R* interval, but unexpectedly increased it in 1-week-old animals. It can be hypothesized that tonic inhibitory effect of the vagus nerve in adult rats is realized through M_3 cholinergic receptors of the heart. The decrease in heart rate during blockade of M_3 cholinergic receptors in 1-week-old rats was probably related to specific innervation of the heart in animals of this age.

Key Words: *heart; M_3 cholinergic receptors; vagus nerve; blood pressure*

Cardiac activity is regulated by the sympathetic and parasympathetic nervous systems. Their influences are realized via interaction with adrenoceptors and muscarinic cholinergic receptors (M-ChR) on cardiac cells [1,8,10]. There are at least 9 subtypes of adrenoceptors and 5 subtypes of M-ChR. M_1-ChR, M_2-ChR, M_3-ChR, M_4-ChR, and M_5-ChR were identified in humans, mammals, and amphibians. Molecular and pharmacological properties of these receptors were extensively studied. Chromosomal localization of various subtypes of human M-ChR was described [4].

It was hypothesized that the chronotropic effect of the vagus nerve includes several components [2,9]. Nonselective blockade of M-ChR with atropine and vagotomy have various effects on the heart [3,6]. According to the general concept, M-ChR of subtype 2 play a major role in parasympathetic regulation of cardiac activity [5,15]. Much attention is paid to the role of M_3-ChR [7,11-14].

The presence of M_1-ChR and M_2-ChR in mammalian myocardium is beyond doubt. The role of M_3-ChR in physiological, pathophysiological, and pharmacological processes in the myocardium remains unknown. Hence, evaluation of age-related differences in the role of M_3-ChR in the regulation of cardiac activity is an urgent problem.

Here we studied the effect of selective M_3-ChR blockade on cardiac activity in rats during early postnatal ontogeny.

MATERIALS AND METHODS

Experiments were performed on 38 rats aging 1, 3, 6, 8, and 20 weeks. Blood pressure (BP) was measured continuously in animals anesthetized by intraperitoneal injection of 25% urethane in a dose of 1000 mg/kg. The right femoral artery was catheterized. The M_3-ChR antagonist 4-DAMP in a single dose of 0.02 mg/kg was administered into the fe-

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moral vein. BP and electrocardiogram (ECG) were recorded continuously and subjected to computer processing. The numerical values of BP, 28 parameters of variational pulsogram, and ECG were estimated using original software.

Five series were performed. In series I, adult animals (20 weeks) received selective M_3-ChR antagonist. These animals have mature system for regulation of cardiovascular activity. DAMP was also injected to rats aging 8 (series II) and 6 weeks (series III). This age corresponds to the period of pubescence. The system of regulation of cardiac activity is unbalanced in animals of this age group. In series IV, the effect of M_3-ChR blockade in 3-week-old rat pups was studied. This period corresponds to the development of sympathetic innervation in the heart. Parameters of cardiac activity after blockade of M_3-ChR in 1-week-old rat pups were evaluated in series V. Sympathetic innervation of the heart is absent in animals of this age.

The significance of differences was evaluated by Student's *t* test and Wilcoxon test (Microsoft Excel).

RESULTS

In 20-week-old rats *R-R* interval decreased from 177.70±32.14 to 149.20±12.05 msec over 1 min after administration of 4-DAMP ($p<0.05$), but returned to 175.10±12.55 msec by the 15th minute postinjection (Fig. 1). Systolic and diastolic BP decreased by 14 and 19%, respectively, 30 sec after M_3-ChR blockade, but by the end of the 15th minute BP surpassed the initial level by 8%, which probably reflected the response to 4-DAMP-induced BP drop (Fig. 2).

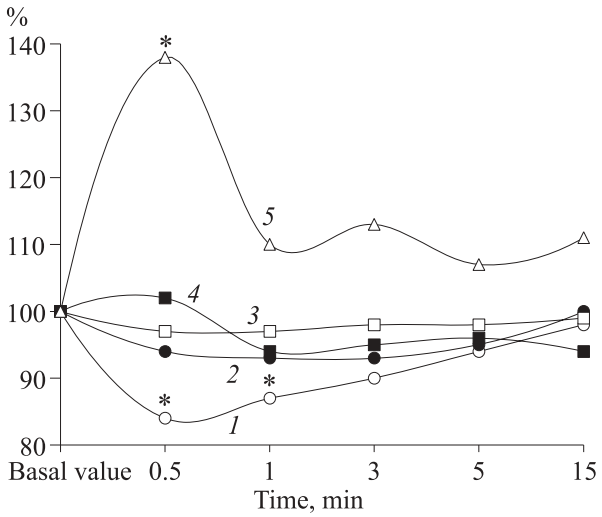

Fig. 1. *R-R* interval in rats of different age after administration of a M_3-ChR antagonist: 20 (*1*), 8 (*2*), 6 (*3*), 3 (*4*), and 1 weeks (*5*). Here and in Fig. 2: **p*<0.05 and ***p*<0.01 compared to the basal value (100%).

Basal *R-R* interval in newborn animals was 241.04±12.00 msec. Administration of 4-DAMP to 1-week-old rats increased *R-R* interval by 38% ($p<0.05$). *R-R* interval returned to normal in the follow-up period and was 257.00±13.74 mec by the 15th minute after 4-DAMP injection (Fig. 1).

In 3-week-old rats, blockade of M_3-ChR had little effect on *R-R* interval: basal value was 132.10±3.52 msec, immediately after 4-DAMP injection, *R-R* interval was 134.60±3.14 msec (Fig. 1), and by the 5th and 15th minutes after treatment 127.20±5.17 and 125.30±4.76 msec, respectively.

In 6-week-old rats, M_3-ChR blockade had minor effect on *R-R* interval: it decreased from 151.30± 6.98 to 146.90±6.38 msec by the 1st minute after

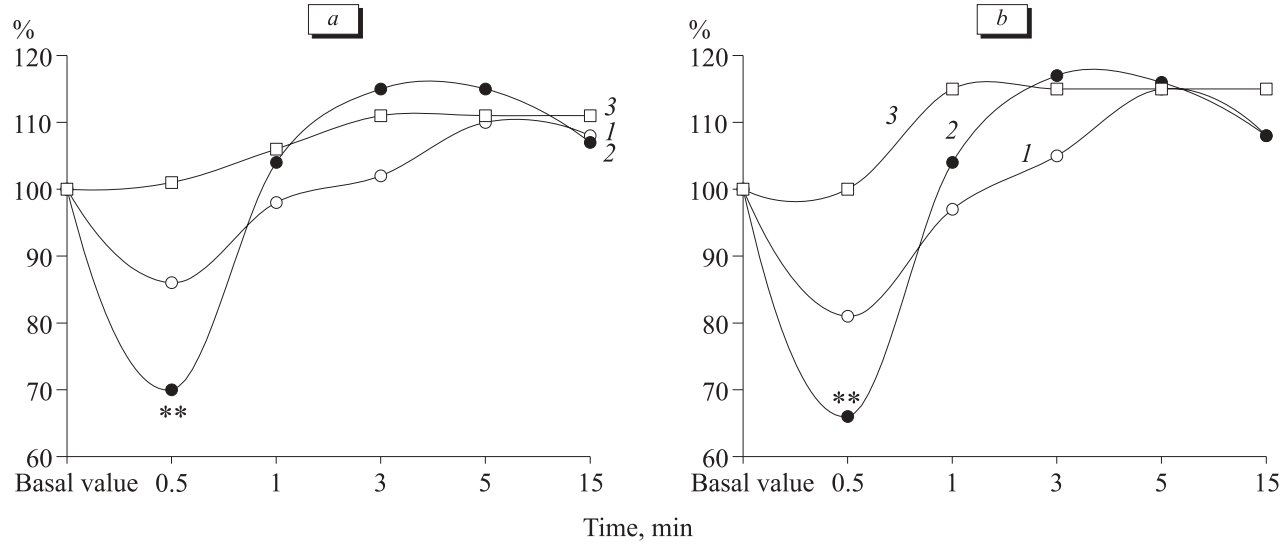

Fig. 2. Systolic (*a*) and diastolic BP (*b*) in rats of different age after administration of a M_3-ChR antagonist: 20 (*1*), 8 (*2*), and 6 weeks (*3*).

treatment. After 15 min, *R-R* interval was 148.60±5.54 msec (Fig. 1). At the same time we observed a gradual increase in systolic (from 93.64±8.46 to 103.60±6.91 mm Hg) and diastolic BP (from 66.76±7.61 to 77.03±8.13 mm Hg) over 15 min postinjection (Fig. 2).

In 8-week-old animals, basal *R-R* interval was 163.1±7.6 msec. Systolic and diastolic BP in these rats was 85.10±4.53 and 72.60±5.21 mm Hg, respectively. Bolus injection of 4-DAMP had little effect on the *R-R* interval in 8-week-old rats. The maximum decrease in this parameter was 7% of the basal level. The *R-R* interval returned to normal by the 15th minute (Fig. 1). However, we observed a significant decrease in systolic and diastolic BP (by 30 and 34%, respectively, $p<0.01$). Systolic and diastolic BP slightly increased in the follow-up period and returned to normal by the 15th minute (90.70±6.82 and 78.61±7.17 mm Hg, respectively; Fig. 2).

Our results indicate that M_3-ChR blockade has a strong effect on the cardiovascular system. Administration of a selective M_3-ChR antagonist is followed by an increase in the heart rate and decrease in BP in adult animals. However, the heart rate in 1-week-old rat pups significantly decreases after this treatment. It can be hypothesized that this population of M-ChR plays different roles in various periods of postnatal ontogeny.

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