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Effect of Clonidine Hydrochloride on Isolated Newborn Rat Heart

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The concentration dependenies of the chronotropic response and changes in blood supply to the isolated heart of 7-day-old newborn rats induced by application of α2-adrenergic receptor agonist clonidine hydrochloride in concentrations of 10^{-9}-10^{-6} M were revealed. The minimum concentration of α2-adrenergic receptor agonist caused tachycardia, while higher concentrations led to bradycardia. The maximum effect manifesting in a decrease in coronary flow was recorded at the minimum concentration of the agonist, while the highest concentration had no effect on the coronary flow. When comparing these results with those obtained in control adult rats, we found that the most pronounced differences in the chronotropic effects were observed after addition of the minimum concentration of the α2-adrenergic receptor agonist: bradycardia in adult rats and tachycardia in newborns. The maximum differences in coronary flow parameters were observed after addition of α2-adrenergic receptor agonist in the maximum concentration that induced a two-phase response in adult rats and had no effect on the blood supply in newborns.

Key Words: *α2-adrenergic receptors; isolated heart; chronotropy; coronary flow; newborn rat*

It was previously believed that only α1- and β-adrenergic receptors (AR) are involved in the regulation of cardiac activity, and this has been shown in most clinical and experimental studies [1,5,13]. As for α2-ARs in the mammalian heart, it was believed until now that they only modulate regulatory influences, being located presynaptically and inhibiting the catecholamines release. Presynaptic inhibition by phentolamine has been shown to be effective in patients with heart failure with increased sympathetic activity [1].

Using PCR, all 3 subtypes of α2-AR were found in the human heart [2]. Radioligand analysis, immunoblotting, and reverse transcription PCR (RT-PCR) also revealed the presence of all subtypes of α2-AR in the myocardium of both normotensive and spontaneously hypertensive rats (SHR) [4]. The presence of α2A-AR, α2B-AR and α2C-AR was shown in the myocardial sarcolemma. In cardiomyocytes, α2-AR activation causes signaling through PI3 kinase and protein kinase B, which leads to an increase in NO production and a decrease in the level of intracellular Ca^{2+} [7,8].

Catecholamine-induced signaling is important for the development of heart cells. α1-AR, α2-AR, and β-AR are possible regulators of cardiomyogenesis [9]. α2-AR play a determinate role in embryonic cardiomyocytes by participating in the actin organization and decreasing cAMP level [12]. Chronotropic effects of selective and nonselective α2-AR blockade in newborn rats were shown. Blockade of α2-AR in newborn rats with yohimbine led to bradycardia [15]. Blockade of α2A-AR and α2B-AR caused tachycardia, while inhibition of α2C-AR had no effect [14]. Blockade of α2A-AR caused a negative inotropic effect in experiments with ventricular myocardium strips from newborn rats [6]. Knockout of α2-AR can lead to animal death, an increase in the heart mass, thickness of

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cardiomyocytes, and changes in activity of signaling pathways [10].

Despite the fact that changes in activity of the sympathetic nervous system are an acknowledged cause of cardiovascular failure, detailed studies of adrenergic interactions in the heart during ontogenesis are insufficient.

Our aim was to study the functional responses of the isolated newborn rat heart to stimulation of α2-AR with clonidine hydrochloride.

MATERIALS AND METHODS

The *ex vivo* experiments were carried out on outbred newborn (7-day-old) albino rats (n=7) lacking sympathetic innervation of the heart. The control group comprised mature rats aging 20 weeks (n=7) with developed system of the autonomic control of the heart. The study was performed in compliance with ethical principles and rules of good laboratory practice.

The rats were anesthetized with 25% urethane (800 mg/kg). The hearts were promptly isolated, washed, and placed in cold Krebs—Henseleit solution (2-5°C). The isolated heart was mounted on a cannula through the aorta and perfused with oxygenated (95% O_2, 5% CO_2) working solution at 37°C and under constant hydrostatic pressure of 60-65 mm Hg using a Langendorff system (AD Instruments). To calculate HR in newborn rats, their cardiac electrograms were led with atraumatic electrodes mounted directly on the heart. The changes in coronary flow (CF) induced by pharmacological agents were also measured. The signals were recorded in a PowerLab 8/35 system (AD Instruments) operated under LabChart Pro software. In the experiments, the α2-AR agonist clonidine hydrochloride (Sigma) was used in concentrations of 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M.

Statistical processing of the obtained results was carried out using Microsoft Excel software; one-factor ANOVA followed by a posteriori test (t test) for related and unrelated groups, and paired and unpaired Student's t tests were applied. The differences were significant at $p<0.05$.

RESULTS

After addition of α2-AR agonist in a concentration of 10^{-9} M to the perfusion solution, HR of the isolated heart of newborn rats was 205.5±16.3 bpm. By the 10th minute, HR increased to 275.5±21.0 bpm ($p<0.01$). By the end of recording (20 min), HR increased by 53% (305.4±12.0 bpm; $p<0.05$) (Figs. 1, 2). In the control group, a significant decrease in HR by 8% ($p<0.05$) was observed (Fig. 2). The CF of the isolated heart of newborn rats after application of 10^{-9} M clonidine decreased 1.54±0.30 to 1.37±0.30 ml/min ($p<0.05$) by minute 10 of the experiment. By minute 20, CF decreased by 26% (to 1.2±0.3 ml/min; $p<0.05$). In the control group of 20-week-old animals, CF decreased by 15% ($p<0.05$) (Fig. 3).

Perfusion of the isolated heart with a solution containing 10^{-8} M clonidine hydrochloride reduced HR from 168.4±13.3 to 149.4±7.1 bpm by the 5th minute of the experiment. The maximum decrease was observed at the 20th minute: 20% from the initial value (to 135.0±6.8 bpm; $p<0.01$) (Fig. 1). In the control group, the decrease in HR was 23% of the initial value. The α2-AR agonist reduced CF of the isolated heart of newborn rats by 25% (from 2.3±0.1 to 1.7±0.3 ml/min). In the control group, opposite effects of α2-AR activation were observed: an increase in CF by 33% ($p<0.05$) with a tendency to recovery and a smooth decrease by 27% ($p<0.05$).

After addition 10^{-7} M clonidine hydrochloride to the perfusion solution, HR decreased by 20% (from 172.5±11.5 to 139.5±16.0 bpm; $p<0.05$) (Fig. 1). In the control, a 20% decrease in HR was observed ($p<0.05$). α2-AR stimulation decreased CF by 20% (from 1.65±0.10 to 1.32±0.10 ml/min) (Fig. 1), while in the control, this parameter increased by 16% ($p<0.01$) and then returned to the initial values.

By minute 15 of perfusion of isolated newborn rat heart with a solution containing 10^{-6} M clonidine hydrochloride, HR decreased by 25% (from 201.8±11.2 to 177.8±9.5 bpm; $p<0.05$) (Fig. 1, 3). In the control group, α2-AR activation induced opposite changes: in some animals, HR decreased by 13% ($p<0.05$), in others, it increased by 9% ($p<0.05$) (Fig. 3). Addition of α2-AR agonist to the perfusion solution did not affect CF in newborn rats (Figs. 1, 3). In the control group, α2-AR activation induced biphasic change in CF: an increase by 9% ($p<0.01$) by minute 1 followed by gradual decrease by 13% ($p<0.05$).

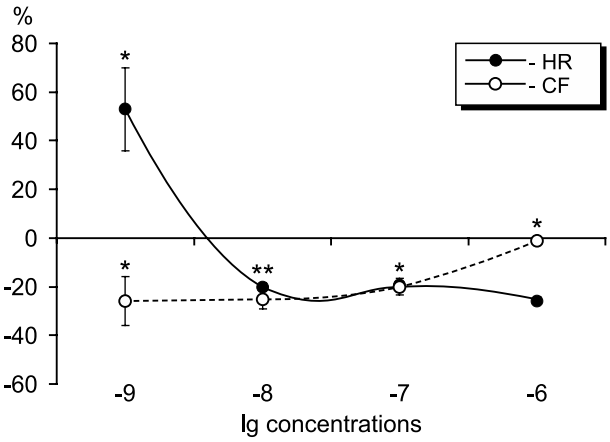

Fig. 1. Effect of clonidine hydrochloride (10^{-9}-10^{-6} M) on HR and CF of Langendorff-perfused isolated newborn rat heart. *$p<0.05$, **$p<0.01$ in comparison with the initial values.

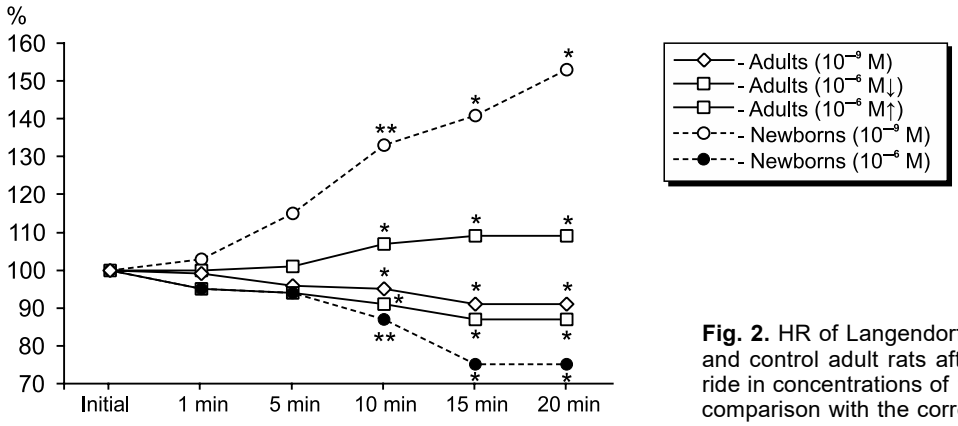

Fig. 2. HR of Langendorff-perfused isolated hearts of newborn and control adult rats after application of clonidine hydrochloride in concentrations of 10^{-9} and 10^{-6} M. *p<0.05, **p<0.01 in comparison with the corresponding initial values.

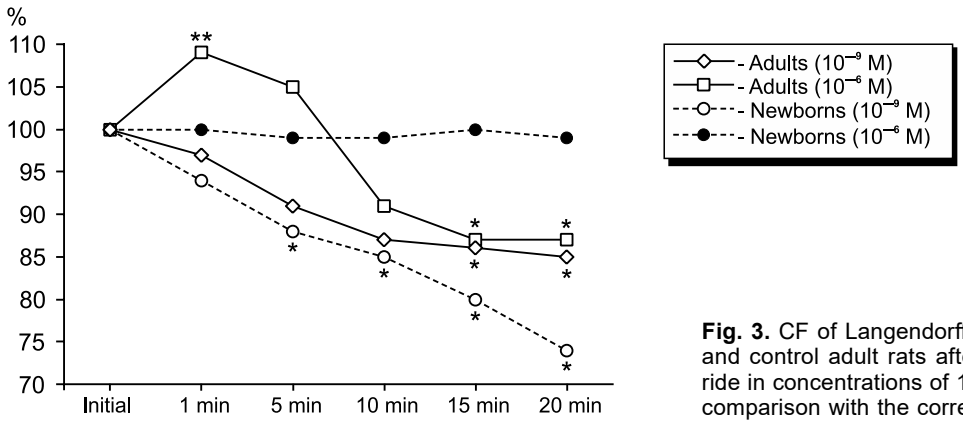

Fig. 3. CF of Langendorff-perfused isolated hearts of newborn and control adult rats after application of clonidine hydrochloride in concentrations of 10^{-9} and 10^{-6} M. *p<0.05, **p<0.01 in comparison with the corresponding initial values.

Despite the fact that final development of the adrenergic innervation of the heart occurs not earlier than 2-3 weeks after birth, cardiomyocytes in rodents respond to adrenergic receptors agonists even in the embryonic period [11]. In addition, prenatal effect of catecholamines is essential for the development of normal heart function [3]. In light of new data on the presence of α2-AR in the cardiomyocyte membrane, information on postnatal changes in α2-adrenergic regulation of cardiac functions is very important. Our studies have demonstrated changes in the studied parameters in the isolated heart of newborn rats after α2-AR stimulation. The chronotropic response of the heart and blood supply to application of clonidine hydrochloride depends on the concentration of α2-AR agonist. The minimum concentration of the agonist caused HR increase, while higher concentrations led to bradycardia. The maximum effect, which was expressed in CF decrease, was recorded after application of the minimum concentration of the agonist; the maximum concentration of clonidine hydrochloride did not affect CF in newborn rats. Comparison of the results obtained in newborns rats with those in the control group (adult rats) showed that the maximum differences in chronotropic effects were observed at minimum concentration of α2-AR agonist that caused bradycardia in adult rats and tachycardia in newborns. The maximum differences in CF parameters were revealed at the maximum concentration of the α2-AR agonist: a biphasic response in adult rats and no changes in newborns.

Thus, we revealed the presence of significant age-related differences in the effect of α2-AR on regulation of cardiac activity. It should be noted that age-related features were observed by us in previous *in vivo* and *in vitro* experiments [6,14,15]. The opposite effects induced by different concentrations of α2-AR agonist can be explained by the participation of different subtypes of α2-AR with different functional activity in the regulation of the heart, as well as differences in presynaptic and postsynaptic α2-AR signal transmission [6-8,14].

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