Cardiac Activity and Blood Pressure in Rats during Selective Blockade of Various Subtypes of Muscarinic Cholinoceptors

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Cardiac activity and blood pressure in adult rats were recorded during selective blockade of cholinoceptors. Blockade of muscarinic M_1 and M_2-cholinoceptors had little effect on cardiac activity. Blockade of muscarinic M_3-cholinoceptors was followed by heart acceleration. The data suggest that the tonic inhibitory influence of the vagus nerve is mediated via cardiac muscarinic M_3-cholinoceptors. Electrostimulation of the right vagus nerve during selective blockade of various subtypes of muscarinic cholinoceptors was followed by the decrease in heart rate. Our results indicate that muscarinic cholinoceptors play a role in the immediate inhibition of cardiac activity upon vagus nerve stimulation.

Key Words: *heart; muscarinic cholinoceptor subtypes; vagus; blood pressure; rats*

There are 5 subtypes of muscarinic cholinergic receptors (AChR) in mammals and amphibians [5,6]. Four types of muscarinic AChR were identified in the human heart [4]. Published data show that parasympathetic regulatory influences on the heart are realized via muscarinic M_2 AChR [10]. Recent studies indicate that muscarinic M_1 and M_3 AChR play a role in cholinergic regulatory influences on the heart [4,11,13,14].

Study of vagal regulation of the heart in laboratory animals revealed significant interstrain differences in the mechanisms of cardiac rhythm regulation in rats [1,3,7]. The possibility of immediate and tonic vagal inhibition of cardiac activity in rats was hypothesized [2]. Age-related and interstrain differences in the autonomic regulation of cardiovascular function are probably related to heterogeneity of musarinic AChR in cardiomyocytes and variations in the density and activity of these receptors. It is important to evaluate the effect of selective blockade of various subtypes of muscarinic AChR.

Here we studied activity of the cardiovascular system in rats during selective blockade of various subtypes of muscarinic AChR.

MATERIALS AND METHODS

Experiments were performed on 29 adult rats. The animals were anesthetized by intraperitoneal injection of 25% urethane (1000 mg/kg). The right vagal trunk was prepared and ligated under a microscope. The hairs were removed and the surgical field was treated with iodine and alcohol. The skin on the inner surface of the thigh was cut, the neurovascular bundle was excided, and the femoral artery was prepared and ligated. A plastic catheter filled with 1% heparin was inserted into the artery through a small transverse section. Another end of the catheter was connected to a device for blood pressure (BP) measurements. We measured systolic (SBP)

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and diastolic BP (DBP). Antagonists of muscarinic M_1 (pirenzepine, Sigma), M_2 (gallamine, Sigma), and M_3 AChR (4-DAMP, Tocris) in a dose of 0.02 mg/kg were administered into the femoral vein (bolus infusion).

An ESL-2 stimulator was used for vagus nerve stimulation (amplitude 5 V, duration 10-12 msec, delay 0.2-0.4 msec, frequency 0.7-10 Hz). The parameters of stimulation were selected individually and remained unchanged during the study. Stimulation of the right vagus nerve was applied over 100 *R-R* intervals.

ECG and BP were continuously recorded. The data were subjected to computer processing. The numerical values of BP, 28 parameters of ECG, and variational pulsogram were obtained using original software.

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

RESULTS

Selective blockade of muscarinic M_1 AChR with pirenzepine had little effect on the mean *R-R* interval (X_m) and BP. Maximum changes were observed 5 min after pirenzepine administration. We revealed a decrease in X_m (Fig. 1). BP decreased 30 sec after pirenzepine administration, but increased by the 3rd minute (Table 1). Heart rate variability remained unchanged during blockade of muscarinic M_1 AChR.

Blockade of muscarinic M_2 AChR with gallamine had little effect on X_m (Fig. 1). BP decreased 30 sec after gallamine administration, but returned to normal by the end of the 1st minute (Table 1).

X_m significantly decreased 30 sec after blockade of muscarinic M_3 AChR with 4-DAMP ($p<0.05$, Fig. 1). Basal heart rate returned to normal over 15 min. BP decreased 30 sec after M_3 AChR blockade, but increased by the 1st minute. BP reached maximum by the 5th minute after treatment (Table 1). Study of heart rate variability showed that the range (dX) decreased 30 sec after M_3 AChR blockade (from 8.43±6.64 to 4.14±3.54 msec), but returned to normal by the 15th minute (7.00±1.34 msec).

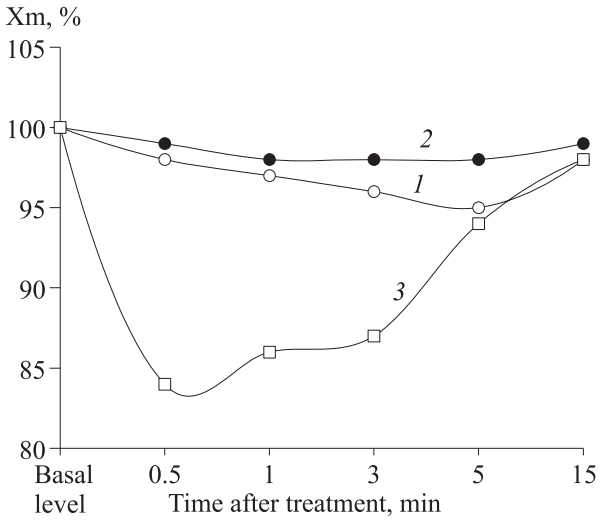

Fig. 1. *R-R* intervals after treatment with selective antagonists of muscarinic M_1 (*1*), M_2 (*2*), and M_3 cholinoceptors (*3*).

We studied the role of each subtype of muscarinic AChR in the immediate vagal inhibition of cardiac activity. The right vagus nerve was stimulated during blockade of various subtypes of muscarinic AChR.

Electrostimulation of the vagus nerve caused bradycardia in intact animals. X_m increased in the early period, but returned to normal by the end of stimulation (Table 2, Fig. 2).

BP decreased during vagus nerve stimulation, but increased after stimulation.

Vagus nerve stimulation during selective blockade of muscarinic M_1 AChR with pirenzepine was accompanied by a significant increase in X_m ($p<0.05$,

TABLE 1. BP in Rats during Blockade of Various Subtypes of Muscarinic AChR (mm Hg, *M±m*)

Parameter	Basal level	Period, min				
		0.5	1	3	5	15
SBP						
M_1 AChR blockade	100.80±6.99	90.6±6.3	103.97±6.76	104.3±17.1	103.03±5.30	98.9±4.2
M_2 AChR blockade	90.86±5.12	80.47±5.51	89.85±5.26	94.65±5.52	93.28±4.56	92.03±5.12
M_3 AChR blockade	91.61±20.56	79.02±25.58	89.68±8.41	93.52±7.35	101.97±9.15	98.38±3.56
DBP						
M_1 AChR blockade	76.98±4.77	71.04±4.40	83.38±5.62	83.48±15.30	83.67±4.40	77.36±4.40
M_2 AChR blockade	72.72±5.57	62.67±6.65	70.14±5.62	74.24±6.33	72.68±6.03	71.29±7.48
M_3 AChR blockade	64.62±15.74	52.87±23.68	62.72±6.27	68.07±5.76	74.97±1.71	71.24±1.65

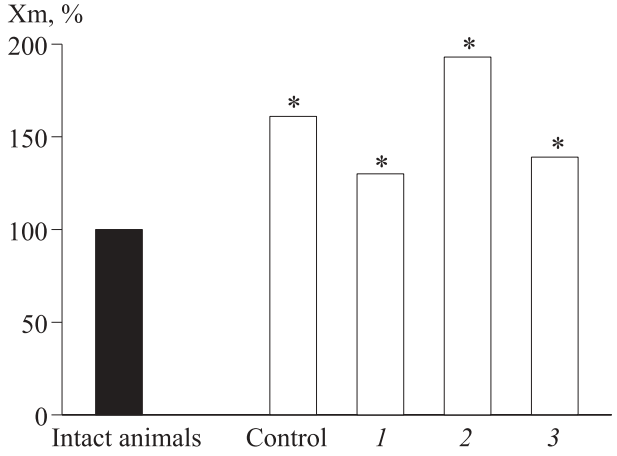

Fig. 2. *R-R* intervals upon electrical stimulation of the vagus nerve before and after blockade of muscarinic M_1 (*1*), M_2 (*2*), and M_3 cholinoceptors (*3*).*p<0.05 compared to initial level.

Fig. 2). X_m returned to normal after stimulation. Stimulation after pirenzepine administration was accompanied by the decrease in BP. BP returned to normal after stimulation (Table 2). The mode amplitude decreased, while dX increased during vagus nerve stimulation.

Electrostimulation of the right vagus nerve during M_2 AChR blockade was accompanied by a considerable increase in X_m (Fig. 2) and insignificant decrease in BP (Table 2). X_m and BP returned to normal after stimulation of the right vagus nerve. Vagus nerve stimulation decreased the mode amplitude, but increased dX. Variational pulsogram returned to normal after stimulation.

Vagus nerve stimulation after 4-DAMP administration was followed by a significant increase in X_m ($p<0.05$, Fig. 2) and decrease in BP. X_m, SBP, and DBP returned to normal after stimulation. Electrostimulation of the right vagus nerve after 4-DAMP administration decreased the mode amplitude, but increased dX. These parameters returned to normal after stimulation.

Previous studies showed that nonselective blockade of muscarinic AChR had no effect on heart rate [2]. Administration of selective antagonists of muscarinic M_1 and M_2 AChR was not accompanied by significant increase in heart rate. By contrast, heart rate significantly increased during selective blockade of muscarinic M_3 AChR. These data provide evidence for the hypothesis that muscarinic M_3 AChR play a role in the cardiac parasympathetic regulation in rats [8,9,12-14]. The short-term decrease in BP after muscarinic AChR blockade reflects the vasoconstrictor effect of acetylcholine. Published data show that administration of atropine prevents bradycardia during electrostimulation of the vagus nerve [2,3]. Selective blockade of 1 of 3 subtypes of muscarinic AChR did not prevent the decrease in HR during stimulation of the right vagus nerve. Our results suggest that the immediate vagal inhibition is mediated by various populations of muscarinic AChR. The decrease in BP during vagus nerve stimulation is probably associated with reduction of the heart rate and impairment of pump function of the heart. The increase in BP after vagus nerve stimulation probably serves as a reflex response.

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TABLE 2. Cardiac Activity during Electrical Stimulation of the Vagus Nerve after Selective Blockade of Muscarinic AChR (*M*±*m*)

Experimental conditions	SBP, mm Hg	DBP, mm Hg	X_m, msec	Mode amplitude, %	dX, msec
Control	83.42±8.97	75.93±8.43	175.1±15.6	47.32±4.76	10.30±2.09
	79.57±5.22	73.19±4.99	283.1±44.1*	27.40±4.17	102.14±43.60
M_1 AChR blockade	95.2±6.2	73.29±3.70	167.50±4.04	44.86±4.02	10.00±2.07
	84.2±8.4	65.27±7.60	217.4±25.2	26.86±4.82**	77.4±45.7
M_2 AChR blockade	83.43±6.91	62.71±7.72	198.50±8.74	43.43±4.81	8.43±1.65
	70.30±8.98	45.68±9.88	386.30±57.71	18.29±2.33	225.86±225.90
M_3 AChR blockade	86.04±6.76	59.66±4.56	178.80±11.50	43.14±4.38	13.14±7.38
	66.24±6.98*	39.08±6.29*	248.50±25.55*	26.00±3.15**	109.86±61.48

Note. Numerator, before vagus nerve stimulation; denominator, after vagus nerve stimulation. *$p<0.05$ and **$p<0.01$ compared to prestimulation parameters.

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