Effects of Low Ethanol Doses on Heart Rhythm in Rabbits

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Effects of low ethanol doses on the vagosympathetic mechanisms of heart rhythm regulation were studied in rabbits. Analysis of heart rhythm variability showed that single intragastric administration of 0.5 mg/kg ethanol caused tachycardia in animals with initial predominance of vagal activity and bradycardia in animals with predominating sympathetic mechanisms. This was associated with general activation of all regulatory effects on the heart rhythm and a drastic increase in power spectrum for all frequency ranges, though with a certain deficiency of vagal effects. However, after 24 h the vagal component of the spectrum drastically increased in animals of both groups, while other parameters did not differ from the control. Presumably, this rebound can be used as a physiological marker, ethanol tolerance measure, formation of the abstinence syndrome and liability to alcoholism.

Key Words: *ethanol; heart rhythm variability; rabbit; vagosympathetic balance*

Effects of ethanol in low doses in various cardiovascular diseases, including heart rhythm disorders, attract much recent attention [2-6,9,10,12,15]. Experimental and epidemiological reports [4,6,10] present data unambiguously confirming the favorable effects of moderate ethanol consumption at all levels of organization of biological systems, from molecular [7,11] to population [14]. However, even very low ethanol doses are harmful, for example, in some tumors or during convalescence after hepatitis C, *etc.*, [15]. Possible mechanisms of these effects remain a topic for discussion, as it is not clear whether these effects are caused by ethanol and its derivative acetaldehyde or by their cooperation with other organic and mineral components of alcoholic drinks [2,4,7,9]. The role of individual and population emotional motivation and social strain associated with personal life style and with the genetic, ethnic, climatic, and other characteristics determining the efficiency of "therapeutic" alcohol doses and the formation of predisposition for alcoholism [3,5,7,14] was never studied. Experimental simulation of these conditions, including simulation in humans, involves great difficulties. However, detection of primary physiological reactions to a single low dose of alcohol can prompt approaches to experimental studies of not only above listed problems, but of the mechanisms triggering the development of alcoholism. The common pattern of activation of the neurovegetative sphere and emotional motivation behavior after a single low dose of alcohol is a measure of the adaptive potentialities and liability of humans or animals to alcoholism [3,11]. The study of the main heart rhythm parameters and their correlations with single ethanol intake in low dose is interesting from the theoretical and practical viewpoints, because the parameters of heart rhythm variability (HRV) can be more informative than just the mean heart rate [3].

We previously studied the effect of single intragastric low dose of ethanol on the rabbit HRV [8], but the time course of changes in the structure of HRV parameters in animals with initially different neurovegetative status has not been studied. Now we investigated the effects of low ethanol doses on heart rhythm of Chinchilla rabbits with initially different vagosympathetic balance and studied changes in the structure of HRV parameters reflecting the vagosym-

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pathetic balance and, presumably, neurovegetative status in general [1].

MATERIALS AND METHODS

Experiments were carried out in autumn-winter on 14 male Chinchilla rabbits (2.5-3.0 kg). The animals were born and bred in the local Breeding Center. At the age of 4 months the rabbits were placed into separate cages in which they were kept during the entire period of observation under the same conditions with free access to food and water. Before the experiments the neurovegetative status of animals was evaluated [1] and they were divided into vagotonics (VT) and sympathotonics (ST). Spectral analysis of HRV included determination of the total power spectral density ($msec^2$), integral powers of spectrum densities for high frequencies ($msec^2$) at 1.7-0.4 Hz taken for the marker of mainly vagal activity, for low frequencies ($msec^2$) at 0.4-0.15 Hz taken for the marker of mainly sympathetic activity, and for very low frequencies ($msec^2$), the genesis and physiological interpretation of which remain disputable.

Analysis of time parameters included pulse rate, mean *R—R* intervals, standard deviation of the mean *R—R* interval, mode at 2 msec step of histogram plotting, mode amplitude, range of *R—R* interval deviations, and R. M. Baevskii's index of strain adapted for rabbits in standardized calculation.

HRV parameters were compared using ANOVA and MANOVA methods. The significance of differences was evaluated using Student's, Fisher's (F), and Wilk's (λ) tests. Such a wide spectrum of analyzed HRV parameters was chosen for solving the concomitant problem of determining the most informative or minimum list of simple tests sufficient for reliable evaluation of the vagosympathetic balance and facilitation of preliminary selection of animals. Other details of the experimental method and technology were described previously [1].

Ethanol (0.5 mg/kg, 40% solution) was administered into the stomach through a nasogastral catheter under local anesthesia with 2% dicaine (3-4 min before intubation). Control animals received the same volume of normal saline (placebo). The study included 3 placebo-ethanol stages with 1-week intervals, quantitative analysis was made using weighed means.

RESULTS

All basal values of HRV parameters in VT and ST rabbits, compared by Fisher's test, except for power spectral density at low and high frequencies (%) differed significantly from each other (Tables 1, 2). A similar picture was observed in multiparametrical comparison of the time and frequency parameters, taken separately and together, by Wilk's λ test (λ=0.001-0.005). These control values of individual parameters of HRV and the entire picture of multiparametrical differences between VT and ST are within the confidence interval of previously published data [1] and confirm the conclusion that the complex of HRV parameters can be used for grouping the rabbits by the vagosympathetic balance, which with certain probability reflects the general neurovegetative status. These two groups of animals principally differ by changes in their mean heart rates and *R—R* equivalent after low doses of ethanol. VT developed relative tachycardia after low doses of ethanol with an appreciable increase in the pulse rate at the stage corresponding to maximum ethanol concentration in the blood (2-3 h), while in ST the pulse rate decreased significantly at this stage. Differences between these groups, were clearly pronounced before ethanol administration, but then leveled and became statistically negligible. After 24 h the initial *R—R* values (and hence, heart rates) were virtually restored in both groups.

It could be expected that after these drastic and opposite changes in the heart rate and *R-R* the values of other time parameters after ethanol intake would change depending on the pulse rate changes in animals of both groups. However, this did not happen: standard deviation of the mean *R—R* interval and the range of its deviations drastically increased in both VT and ST, while the mode amplitude and strain index decreased significantly. Significant differences between these parameters in VT and ST rabbits persisted during all periods of observation. Considering the possible changes in the vagosympathetic balance and its internal structure, these changes in the time parameters cannot be interpreted unambiguously. If the development of tachycardia in VT is associated with ethanol suppression of the vagus tone [12], the development of bradycardia in ST can by no means be due to this factor. We should like to emphasize that complex comparison of the pattern of all time parameters by the λ test shows that the significant differences between VT and ST are retained during all stages of observation, though they are less pronounced than in the control.

Spectral characteristics are changing in the same direction: 1-3 h after administration of a low dose of ethanol the total spectral power density and integral values of power spectra in all frequency bands drastically increased in both groups, while specific contribution of each of them is changed negligibly (Table 1). One-two hours after ethanol intake only the percentage of the integral spectral power density at high frequencies decreased insignificantly in animals of both groups compared to the control. Due to this, the differences between all parameters in VT and ST were

somewhat leveled. However, multiparametrical comparison of all spectral characteristics (λ test) still showed statistically significant differences, which was explained by the gradient of changes in individual parameters compared to the control. For example, at different stages after ethanol administration the total power of spectrum density in VT rabbits increased 3-3.5 times *vs.* 7-8-fold in ST rabbits. For low frequencies these values were 3.5-4 and 6-7-fold, for high frequencies 2-2.5 and 3-4-fold, respectively. The percentage of integral power of spectrum density at high frequencies increases in animals of both groups as early as 3 h after the dose intake, while after 24 h this increase was significant ($p<0.05$ *vs.* the control), though there was no significant difference between VT and ST.

Low ethanol doses caused activation of all regulatory effects on the heart rhythm, which was more pronounced in ST. Activation of the vagus tone was somewhat delayed. Moreover, the percentage of integral power of the spectrum density at high frequencies taken as a marker of parasympathetic effects on the heart rhythm changed specifically: a rebound was observed. No difference in this parameter between VT and ST was observed after 24 h, though rebound in the former case surpassed the initial level by 57% and in the latter by 33%. On the other hand, the absolute power of spectrum density at high frequencies during this period was in VT significantly higher than in controls, while in ST complete recovery of this parameter was observed.

If we consider the neuroanatomical and functional relationships between the central parasympathetic structures in the organization of emotional motivation and common adaptive behavior and some other assumptions of the polyvagal theory [12], we must admit that along with changes in the heart rate [3], vagus activity rebound 24 h after a low ethanol dose can be an additional and even more informative physiological marker, a measure of ethanol tolerance, formation of

TABLE 1. Time Parameters of Rabbit HRV (VT as Numerator, ST as Denominator) before and after Intragastric Administration of Ethanol (*M±m*)

Parameters	Control	Time after intragastric administration of ethanol, h			
		1	2	3	24
Pulse rate/min	235.2±3.6 / 293.6±5.0	243.3±6.5 / 283.8±3.6	26+1.7±5.6* / 275.0±4.2	281.0*±11.8 / 276.0±5.0	242.5±7.3 / 282.6±3.5
	pF<<0.001	pF<<0.001	pF>0.12	pF>0.42	pF<0.001
R—R, msec	254.9±4.0 / 204.3±3.5	246.6±6.3 / 211.4±2.6	229.3±4.9* / 218.1±3.4	213.5±9.0* / 217.4±4.0	247.4±7.6 / 212.3±2.7
	pF<<0.001	pF<<0.001	pF>0.86	pF>0.29	pF<<0.001
Standard deviation of the mean *R—R*, msec	7.7±0.5 / 3.0±0.2	15.7±2.0* / 6.0±0.5**	14.5±2.1* / 6.5±0.6**	12.4±2.0 / 5.4±0.4**	8.0±0.6 / 3.5±0.3
	pF<<0.001	pF<<0.001	pF<<0.001	pF<0.016	pF<<0.001
Mode amplitudes, %	14.6±0.8 / 29.5±1.6	8.7±1.0** / 18.1±1.5**	9.4±1.1** / 18.1±2.1**	13.3±2.0 / 21.2±1.4*	14.1±1.3 / 27.4±1.8
	pF<<0.001	pF<0.001	pF<0.01	pF<0.016	pF<<0.001
Range of *R—R* interval deviations, msec	37.1±0.4 / 15.2±0.2	69.2±6.1** / 28.2±2.3**	61.3±6.4* / 30.5±2.8**	55.0±6.1 / 25.6±1.6**	39.2±3.0 / 17.7±1.5
	pF<<0.001	pF<<0.001	pF<0.002	pF<<0.001	pF<<0.001
Strain index	3.4±0.4 / 11.0±1.1	1.1±0.2** / 4.7±1.0*	1.5±0.4* / 5.3±1.4*	2.4±0.6 / 5.2±0.9*	2.9±0.6 / 10.5±1.2
	pF<<0.001	pF<0.001	pF<<0.001	pF<0.05	pF<<0.001
MANOVA	λ=0.0025	λ=0.03	λ=0.06	λ=0.04	λ=0.05

Note. Here and in Table 2: *$p<0.01$, **$p<0.001$ compared to the control.

TABLE 2. Rabbit HRV Frequency Parameters (VT: Numerator; ST: Denominator) before and after Intragastric Ethanol Intake (*M*±*m*)

Parameters	Control	Time after intragastric administration of ethanol, h			
		1	2	3	24
Spectrum density power, msec^2					
total	85.8±22.7	267.6±71.5	298.9±77.8	179.5±26.4	90.2±23.2
	21.9±2.8	155.9±16.4**	167.0±30.1*	110.2±25.4*	17.3±1.5
	pF<0.003	pF>0.6	pF=0.05	pF>0.25	pF<0.001
at very low frequencies	38.8±8.5	137.4±36.6	138.3±30.4*	74.7±14.7	32.7±10.5
	6.6±1.3	2.9±12.5**	83.1±18.3*	42.6±9.5	5.8±1.0
	pF<0.038	pF>0.47	pF<0.03	pF>0.16	pF<0.004
at low frequencies	21.0±4.5	54.6±14.4	73.1±18.7	56.1±10.5*	27.8±8.1
	5.3±0.8	33.9±5.6*	30.9±4.3**	20.6±4.9*	3.9±0.3
	pF<<0.001	pF>0.8	pF<0.016	pF=0.04	pF<0.008
at high frequencies	7.6±1.2	11.9±2.4	16.7±3.6	17.8*±2.5	13.9±2.2
	2.7±0.2	7.3±0.9**	9.3±1.4**	7.8±0.8**	2.7±0.2
	pF<<0.001	pF>0.11	pF>0.08	pF<0.02	pF<0.001
Low frequencies/high frequencies	2.7±0.2	4.6±0.5*	4.4±0.4	3.6±0.4	2.1±0.2
	2.0±0.2	4.7±0.4**	3.3±0.3*	2.6±0.3	1.6±0.1
	pF<0.001	pF>0.42	pF>0.4	pF>0.62	pF<0.03
Low frequencies, %	44.7±2.1	43.4±6.2	47.3±5.9	48.9±5.0	42.6±3.7
	38.0±3.0	42.2±4.5	38.8±4.5	33.6±6.3	34.8±4.2
	pF>0.07	pF>0.31	pF>0.2	pF>0.14	pF>0.16
High frequencies, %	16.1±1.3	11.0±1.2	11.6±0.8	17.0±1.7	25.2±2.8
	17.9±1.5	9.2±0.9**	11.3±1.0**	15.7±4.2	23.9±1.9
	pF>0.48	pF>0.8	pF>0.1	pF>0.58	pF>0.7
MANOVA	λ=0.05	λ=0.42	λ=0.06	λ=0.16	λ=0.08

the abstinence phenomenon and liability to alcoholism. These problems deserve further investigation with consideration for the species specificity of dose dependence, significance of the initial vagosympathetic balance from the viewpoint of evaluating the contribution of total metabolic and circulatory activation [13], on the one hand, and direct effect of ethanol on the pacemaker and central and peripheral nervous mechanisms of heart rhythm regulation, on the other.

Selection of volunteers by psychophysiological characteristics is a common scientifically-based requirement to biomedical studies on humans. When the same problems are solved in animal experiments, this approach rarely used. It seems that the traditional criteria of selection of objects of investigation (species, genetic strain, age, weight, sex, *etc.*) are not always sufficient for practical studies in experimental biology and medicine. Selection by the HRV parameter is sufficiently correct, noninvasive, and operative [1,8].

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