DISTURBANCE OF SYMPATHETIC CONTROL OF CARDIOVASCULAR FUNCTIONS IN RATS WITH STREPTOZOCIN-INDUCED DIABETES

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Experimental diabetes causes considerable changes in the parameters of the systemic and regional hemodynamics in rats [4, 11, 13]. The causes of these changes may be disturbances of nervous control of the functions of the cardiovascular system due to the development of diabetic neuropathies [6, 15], and also changes in the contractile properties of the myocardium and smooth-muscle cells of the vessels [7, 10].

The aim of this investigation was to study the reactivity of the cardiovascular system of rats with streptozocin-induced diabetes to endogenous noradrenalin (NA) by measuring the parameters of the systemic and regional hemodynamics in conscious rats by the isotope-labeled microspheres method.

EXPERIMENTAL METHOD

Secretion of endogenous NA was produced by intravenous injection of the sympathomimetic tyramine, causing release of NA from sympathetic nerve endings [8]. Diabetes was induced in male Wistar rats weighing 350-400 g by a single intravenous injection of streptozocin ("Upjohn," USA) in a dose of 50 mg/kg. Rats of the same age, receiving injections of 0.5 ml of the solvent (citrate buffer, pH 4.5) served as the control. Animals whose blood glucose level 24 h after injection of streptozocin exceeded 300 mg% were considered to be "diabetics." The blood glucose concentration was measured by a glucometer ("Miles," USA). Experimental (diabetic) and control animals were kept separately with free access to food and water. The animal was weighed 13 weeks after injection of streptozocin and used in the experiment. The cardiac output (CO) and blood flow in 14 organs were measured by means of isotope-labeled microspheres by the method described previously [1]. Tyramine was injected into the jugular vein by a peristaltic pump ("Rabbit," USA) in doses causing elevation of the systemic BP by 15-20 mm Hg. The concentration of tyramine solution injected was 0.001 g/ml and the rate of injection varied from 0.025 to 0.14 ml/min. Injection of physiological saline at that rate caused no changes in parameters of the hemodynamics in the control animals or rats with diabetes. The duration of injection was 5 min. Values of CO and blood flow were calculated by standard formulas on a "Labtam-3015" computer (Australia). The results were subjected to statistical analysis by the use of a modified Student's test for groups. Differences were considered to be significant at the $p < 0.05$ level in two directions. The results are given in the form $M \pm m$.

EXPERIMENTAL RESULTS

Animals with diabetes had a much reduced body weight (286.7 ± 12.3 g compared with 385.0 ± 19.5 g in the control), and also a raised blood glucose level (more than 500 mg%). Parameters of systemic and regional hemodynamics at the beginning of the experiment and after injection of tyramine in both groups of animals are given in Table 1.

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TABLE 1. Hemodynamic Parameters in Control Animals and Diabetic Rats before and during Injection of Tyramine

Parameter	Control animals (n = 8)		Rats with diabetes (n = 6)	
	I	II	I	II
Average BP, mm Hg	107,8±3,7	122,0±4,4+	97,0±3,3*	115,0±2,6+
Heart rate, beats/min	407±13	435±20	388±24	423±18
CI, ml/min/100 g	38,7±1,6	55,0±3,3+	44,0±0,7*	64,2±4,1+
SV, ml/beat	0,37±0,02	0,48±0,03+	0,33±0,01	0,43±0,03+
TPVR, mm Hg·ml^{-1}·min^{-1}/100 g	2,80±0,09	2,29±0,19+	2,21±0,06*	1,82±0,08+
Regional blood flow, ml·min^{-1}·g^{-1}:				
skin	0,18±0,02	0,19±0,01	0,18±0,03	0,23±0,03
skeletal muscles	0,11±0,01	0,16±0,02+	0,20±0,04*	0,26±0,03
stomach	1,49±0,19	0,87±0,12+	1,15±0,10	0,88±0,11
pancreas	1,83±0,27	1,27±0,22	1,49±0,26	1,08±0,09
brain	1,54±0,09	1,84±0,14	1,46±0,09	1,94±0,15+
small intestine	2,61±0,23	2,65±0,20	2,58±0,44	2,12±0,35
lungs	1,71±0,25	5,62±1,51+	2,63±0,86	2,25±0,44
spleen	2,44±0,23	1,83±0,12+	3,00±0,46	1,22±0,21+
liver	0,20±0,04	0,20±0,04	0,32±0,05	0,58±0,07+
heart	7,31±0,49	13,76±1,26+	8,51±0,71	13,44±0,96+
adrenals	7,55±0,95	4,97±0,71+	9,89±1,36	8,25±1,94
testes	0,53±0,04	0,42±0,05	0,35±0,03*	0,24±0,03+
kidneys	8,37±0,49	6,85±0,31+	6,03±0,54*	5,29±0,79
diaphragm	1,13±0,10	1,42±0,15	1,13±0,10	1,37±0,14

Legend. I) before, II) after injection of tyramine. +) $p < 0.05$ compared with initial level (before injection of tyramine); *) $p < 0.05$ compared with control.

The doses of tyramine necessary to raise the systemic BP by 15-20 mm Hg differed significantly ($p < 0.05$) in the groups of animals tested: in diabetic rats, to raise BP by 15-20 mm Hg, tyramine was required in a concentration of $(5 \pm 0.4) \cdot 10^{-5}$ g/min/100 g body weight, compared with $(9 \pm 0.89) \cdot 10^{-6}$ g/min/100 g in the control.

Injection of tyramine caused a significant rise of BP in both groups of animals. The parameters of the systemic and regional hemodynamics showed significant changes (Table 1). In the control animals and diabetic rats the cardiac index (CI), the ratio of CO to 100 g body weight, was significantly increased by 42 and 46% respectively, the stroke volume (SV) of the heart was increased by 30% in both groups, whereas the total peripheral vascular resistance (TPVR) was significantly lowered – by 18% in both groups. The blood flow in the organs of the animals of both groups changed in a similar manner: it increased in the thoracic organs, decreased in the abdominal organs (except the liver), and also increased in the skeletal muscles and brain (Table 1).

Changes in resistance of the vessels of different organs in response to injection of tyramine are indicated in Fig. 1.

Attention is drawn to be much stronger pressor response of the splenic vessels of the diabetic rats to injection of tyramine (resistance rose by 184%; $p < 0.05$) compared with the control animals (resistance rose by 40%; $p < 0.05$).

A study of the parameters of the systemic and regional hemodynamics in conscious rats with streptozocin-induced diabetes showed a decrease in BP, an increase in CI, and a decrease in TPVR compared with the age control, as well as changes in the blood flow in a number of vascular regions.

Systemic administration of tyramine to the control animals caused a significant increase in BP. This was accompanied by a significant rise of CI and a significant fall of TPVR. Other workers also have observed a similar response [9]. The increase in CI was due to quickening of the heart rate and a significant increase in SV. This can be explained by direct activation of the myocardium by NA, secreted from sympathetic nerve endings, and also by an increase in the venous return of the blood as a result of contraction of capacitive vessels [3]. At the same time TPVR was reduced due to vasodilatation in the skeletal muscles, heart, and lungs, probably reflecting the appearance of a depressor cardiovascular reflex arising in response to an adrenergic increase in myocardial contractility [2, 14]. Meanwhile vasoconstriction was observed in the abdominal organs, possibly on account of differences in the degree of involvement of the arteries in different regions of the body in the reflex response. Considering that reflex vasodilatation is due to inhibition of sympathetic influences on blood vessels (passive component) and, possibly, to the action of vasodilators (active component) [14], and also that secretion of NA takes place under the influence of tyramine, it can be tentatively suggested that the simultaneous effect of these processes on vascular smooth muscle gives rise to divergent changes in the regional blood flow and resistance, revealed by injection of tyramine into conscious rats. Activation of the baroreflex mechanism as a result of the action of the raised BP on the sinoaortic zone also is possible.

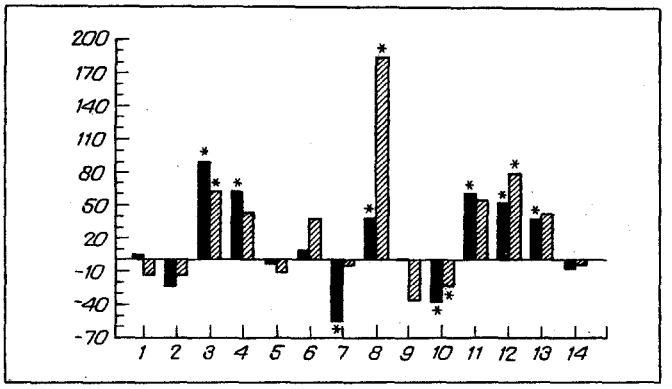

Fig. 1. Changes in regional vascular resistance of control animals and rats with diabetes receiving injections of tyramine. Abscissa: 1) skin; 2) skeletal muscles; 3) stomach; 4) pancreas; 5) brain; 6) small intestine; 7) lungs; 8) spleen; 9) liver; 10) heart; 11) adrenals; 12) testes; 13) kidneys; 14) diaphragm; ordinate, changes in regional vascular resistance (in percent of normal values, before injection of tyramine). Black columns represent control animals, obliquely shaded – rats with diabetes. *$p < 0.05$ compared with initial level (before injection of tyramine).

The response of the cardiovascular system of the diabetic animals to systemic administration of tyramine was similar to that observed in the control rats. BP rose on account of an increase in CI accompanied by reduction of TPVR. This response also presupposes direct activation of the myocardium and reflex vasodilatation in certain regions (skeletal muscles, heart, liver) with simultaneous vasoconstriction of the abdominal organs (except the liver).

The reason why a much larger (by 5.6 times) dose of tyramine is needed for this purpose may be as follows: depressed reactivity of the myocardium in diabetic rats to NA [10] as a result of a decrease in the population of highly sensitive β_1-adrenoreceptors [12], the presynaptic noradrenergic deficit [6, 15], and the greater sensitivity of the baroreflex [5].

The almost twofold increase in resistance in the spleen of animals with diabetes may be due either to weakening of reflex vasodilatation or to increased reactivity of the vessel to NA compared with the control animals.

It can be concluded that disturbances of sympathetic regulation of functions of the cardiovascular system, induced by the diabetic process, may be among the causes of the change in parameters of the systemic and regional hemodynamics in rats with chronic streptozocin-induced diabetes.

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LITERATURE CITED

1. O. S. Medvedev, A. N. Murashev, F. E. Meertsuk, and S. F. Dugin, Fiziol. Zh. SSSR, **72**, No. 2, 253 (1986).
2. A. A. Moibenko, Cardiogenic Reflexes and Their Role in Regulation of the Circulation [in Russian], Kiev (1979).
3. T. D. Bennett, C. R. Wyss, and A. M. Scher, Circulat. Res., **55**, No. 4, 440 (1984).
4. L. F. Carbonell, M. G. Salom, J. Garcia-Estan, et al., Am. J. Physiol., **252**, H900 (1987).
5. K. S. K. Chang and D. D. Lund, J. Molec. Cell. Cardiol., **18**, No. 6, 617 (1986).
6. R. A. Cohen, B. Tesfamariam, R. M. Weisbrod, and K. M. Zitney, Am. J. Physiol., **259**, No. 1, H55 (1990).
7. J. J. Friedman, Am. J. Physiol., **256**, H1134 (1989).
8. B. R. Frost, D. B. Frewin, and J. A. Downey, Eur. J. Pharmacol., **67**, 85 (1980).
9. C. Haracal, R. W. Sevy, and B. F. Rusy, J. Pharmacol. Exp. Thor., **144**, No. 1, 89 (1964).
10. D. J. Paulson, S. J. Ropp, J. P. Tow, and D. G. Peace, J. Pharmacol. Exp. Ther., **240**, No. 2, 529 (1987).
11. A. A. Quyymi, J. L. Iaffaldano, C. A. Guerrero, et al., Diabetes, **38**, No. 12, 1585 (1989).
12. S. Ramanadham and T. E. Tenner, Eur. J. Pharmacol., **136**, 377 (1987).
13. R. G. Tilton, K. Chang, G. Pugliese, et al., Diabetes, **38**, No. 10, 1258 (1989).

14. A. J. Tobia, M. D. Adams, T. S. Miya, and W. F. Bousquet, J. Pharmacol. Exp. Ther., **175**, No. 3, 619 (1970).
15. C. E. E. M. Van der Zee, M. Van don Buuse, and W. H. Gispen, Eur. J. Pharmacol., **177**, 211 (1990).

ELECTRICAL INSTABILITY OF THE HEART INDUCED BY β-ADRENERGIC DAMAGE AND ITS PREVENTION BY THE ANTIOXIDANT IONOL

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KEY WORDS: adrenergic damage; heart; contractile function; electrical stability

Stress-induced injuries, adrenergic in nature, are of great importance in the mechanisms of cardiac arrhythmias and fibrillation [10, 12]. An important role in the realization of these mechanisms is played by catecholamine-induced activation of lipid peroxidation [2, 10]. In recent years stress injuries of the heart have occupied an important place in clinical cardiology [1, 8, 9], for stress is often the cause of arrhythmias and of sudden cardiac death [7-9]. Because of the importance of this problem, the relationship between disturbances of electrical stability of the heart and its contractile function in β-adrenergic lesions, and the possibility of preventing these disturbances by cardioprotective drugs, especially antioxidants, has become an urgent topic for research.

The aim of this investigation was to compare the contractile function and electrical stability of the heart in adrenergic damage, produced with the aid of the well-known β-adrenoreceptor agonist isoprenaline [4, 11]. Isoprenaline, like catecholamines, has an arrhythmogenic action [5] and causes activation of lipid peroxidation [6]. Another aim of the investigation was therefore to study the possibility of preventing isoprenaline-induced damage by using the synthetic antioxidant ionol (butylated hydroxytoluene).

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-270 g. The animals were divided into five groups: 1) control; the animals of groups 2 and 3 were given a subcutaneous injection of isoprenaline in a single dose of 10 and 40 mg/kg respectively; animals of groups 4 and 5 were first given the synthetic antioxidant ionol (per 08), dissolved in sunflower oil (0.05 ml/100 g body weight) in a dose of 30 mg/kg daily for 3 days, and again on the 4th day, 1 h before injection of isoprenaline in the same doses. Acute experiments under pentobarbital anesthesia (50 mg/kg) were carried out 24 h and 30 days after injection of isoprenaline. In the first stage of the experiments the response of the heart to electrical stimulation of the peripheral end of the divided vagus nerve by square pulses (frequency 20 Hz, duration 2 msec, delay 5 msec) by means of an ÉSL-2 electrostimulator was studied. After determination of the threshold value of the stimulus, which in the groups compared varied from 0.17 to 0.21V, the response to stimulation with a strength of 1, 2, 3, and 4 thresholds was assessed successively, with an interval of 5 min. During 30 sec of electrical stimulation, the ECG was recorded in lead I to assess the degree of bradycardia and the character of the arrhythmias and of conduction. Next, under open chest conditions

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