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

L. M. Belkina, R. N. Kolarova, and F. Z. Meerson

UDC 616.12-008.318-085.23-036.8-07

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

Laboratory of Pathophysiology of the Heart, Research Institute of General Pathology and Pathophysiology, Academy of Medical Sciences of the USSR, Moscow. Department of Pathophysiology, Medico-Biological Institute, Medical Academy, Sofia, Bulgaria. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 11, pp. 465-467, November, 1991. Original article submitted September 15, 1989.

0007-4888/91/0011-1549$12.50

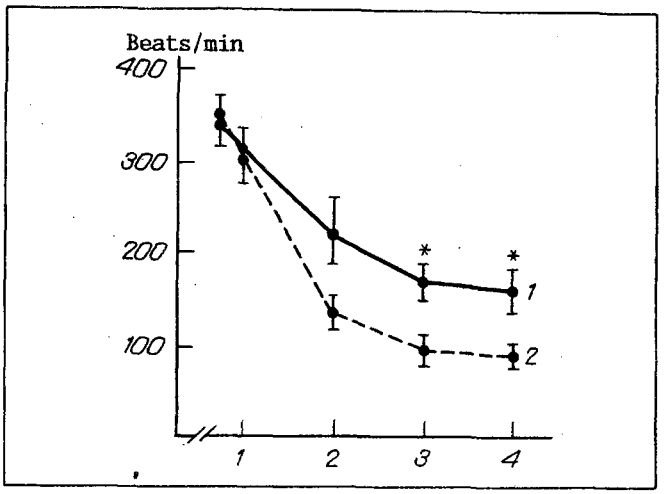

Fig. 1. Effect of vagus nerve stimulation on HR in control animals (1) and 24 h after injection of isoprenaline in a dose of 10 mg/kg (2). Abscissa, strength of stimulation of vagus nerve (in threshold units); ordinate, HR (beats/min). *$p < 0.05$.

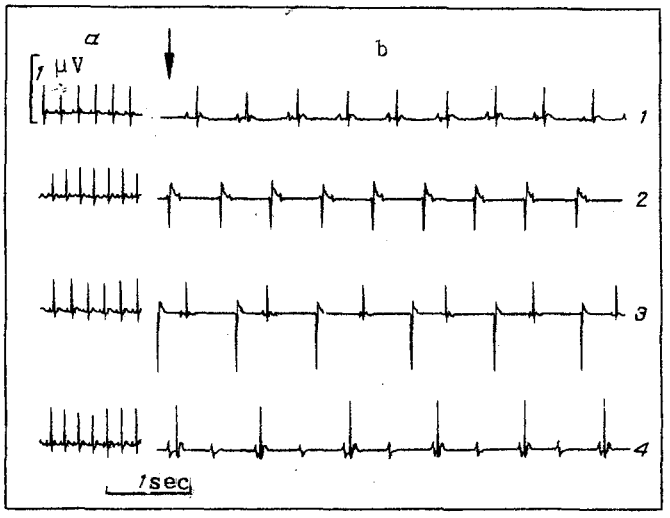

Fig. 2. Typical arrhythmias during vagus nerve stimulation. a and b) ECG in lead I before and during vagus nerve stimulation respectively in control animals (1) and 24 h after injection of isoprenaline in a dose of 10 mg/kg (2-4). Arrow indicates beginning of stimulation. Explanation in text.

and with artificial ventilation, the threshold of ventricular fibrillation (TVF) was determined by the method in [3]. TVF was determined only in rats receiving isoprenaline in a dose of 10 mg/kg, but in animals receiving the drug in a dose of 40 mg/kg this was impossible because of the formation of foci of necrosis in the apical region of the heart. At the second stage of the experiments, also under open chest conditions, contractility of the heart was studied at rest and during maximal isometric loading, produced by ligating the ascending arch of the aorta for 60 sec. The following parameters were determined from the curve of pressure recorded in tho left ventricle by means of a "Mingograf-34" electromanometer ("Siemens-Elema," Sweden): The heart rate (HR), systolic, diastolic, and developed pressures, the intensity of functioning of structures (IFS — the product of the developed pressure and HR, divided by the mass of the left ventriclo); the maximal

TABLE 1. Effect of Vagus Nerve Stimulation on Frequency of Arrhythmias and Character of Rhythm Disturbances in Rats 24 h after Injection of Various Doses of Isoprenaline

Experimental conditions	Number of animals with arrhythmias				
	AV block	idioventricular rhythm	extrasystoles	bradyarrhythmia	total number of animals with arrhythmias
Control	0	0	2 (5)	0	2
Isoprenaline:					
10 mg/kg	2	4	2 (10)	2	7
40 mg/kg	6	4	4 (23)	2	9

Legend. Each group contained nine animals: in the "extrasystoles" column the total number of extrasystoles arising during 30 sec of vagus nerve stimulation is given in parentheses.

rate of development and of fall of pressure, which reflects the velocity of contraction and relaxation of the myocardium respectively. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

No deaths were observed after injection of isoprenaline in a dose of 10 mg/kg, but with a dose of 40 mg/kg mortality was 11%. The absolute weight of the heart after injection of isoprenaline in both doses was increased by 15%. HR in the control animals was 344 ± 12 beats/min, whereas after injection of isoprenaline in doses of 10 and 40 mg/kg it was 358 ± 15 boats/min ($p > 0.1$) and 380 ± 12 beats/min ($p < 0.05$). When the ECG was recorded in a state of relative physiological rest, no arrhythmias or conduction disturbances were found after injection of isoprenaline in both doses.

The study of the response of the heart to vagus nerve stimulation showed that both doses of isoprenaline more than doubled the degree of vagus bradycardia compared with the control (Fig. 1). This points to the onset of a unique syndrome of sinus node weakness in isoprenaline-induced injury.

The main result of these experiments, however, is that various conduction blocks and ectopic rhythms arise in animals receiving injections of isoprenaline. Typical ECGs recorded during inhibition of sinus node function are illustrated in Fig. 2. Unlike in the control (Fig. 2, 1b), in which only sinus bradycardia occurred in response to vagus nerve stimulation, in animals with isoprenaline-induced injury a rhythm from the atrioventricular (AV) junction was observed; excitation spread, moreover, 80 that the ventricles were excited before the atria (Fig. 2, 2b). In another case an idioventricular rhythm was recorded, alternating with the sinus rhythm from the AV junction (Fig. 2, 3b). Finally, on the 4th ECG a block of the I degree can be seen (2:1 rhythm), when atrial complexes occur in regular sequence, and each second ventricular complex is missing. Table 1 shows the frequency of the different types of arrhythmias during vagus bradycardia. Clearly idioventricular rhythms are commonest, AV blocks are observed less frequently, and bradyarrhythmias less frequently still. With an increase in the dose of isoprenaline to 40 mg/kg the frequency of the arrhythmias described above increased, with the exception of bradyarrhythmias. Preliminary injection of ionol completely prevented vagal arrhythmias in animals receiving isoprenaline in doses of 10 mg/kg, but only partially in animals receiving 40 mg/kg. The further study of the character of the disturbances of electrical stability of the heart showed that in animals receiving isoprenaline in a dose of 10 mg/kg vulnerability to arrhythmias was increased, as shown by the fact that TVF in these animals was half as high as in the control (3.1 ± 0.21 and 7.5 ± 0.24 mA respectively; $p < 0.001$). This effect was completely prevented by ionol. Disturbances of electrical stability of the heart thus revealed were reversible in character, and had completely disappeared 30 days after injection of isoprenaline.

The results are evidence that isoprehaline, in moderate doses, can cause latent disturbances of electrical stability of the heart, manifested as a syndrome of sinus node weakness, conduction disturbances, abnormal activation of cardiomyocytes, and increased risk of fibrillation.

Comparison of disturbances of electrical stability and parameters of contractility of the heart showed that in the intact animal neither dose of isoprenaline caused disturbances of cardiac function whether at rest or during maximal isometric work. Thus after 60 sec of compression of the aorta. HR in the control group and in animals receiving isoprenaline in a dose of 10 mg/kg was 216 ± 7 and 258 ± 6.7 beats/min ($p < 0.05$) respectively, the developed pressure was 209 ±

6.7 and 199 ± 9.3 mm Hg ($p > 0.1$), and the diastolic pressure was 21 ± 3.3 and 12 ± 2.1 mm Hg respectively ($p < 0.05$). It will be noted that in animals with isoprenaline-induced injuries HR was increased during this period, but the diastolic pressure was significantly reduced. This led to the fact that the product of HR and developed pressure in these animals was actually higher than in the control (45, 144 ± 720 and 51, 342 ± 1147 mm Hg respectively; $p < 0.001$). However, if this parameter was calculated per unit of mass of the myocardium, the difference between the groups disappeared, for after injection of isoprenaline the mass of the left ventricle rose by 15% compared with the control. As a result, the value of the parameter IFS was similar in the groups compared, and in the control and in animals receiving isoprenaline it was 89 ± 7 and 88 ± 6 mm Hg·n/mg respectively (where n denotes HR). These data indicate that in the intact animal local disturbances of myocardial contractility, associated with the formation of microfoei of necrosis under the influence of isoprenaline [11] are compensated by the function of the undamaged myocardium.

Thus in β-adrenergic damage a unique kind of dissociation is observed between the increased electrical stability and the undamaged contractile function of the whole heart. When this phenomenon is analyzed it must be recalled that microfocal lesions of the myocardium, not affecting the function of the whole heart, may nevertheless cause disturbances of conduction and of impulse generation. These phenomena were observed in the experiments described above. Essentially, disturbances of this kind may be prevented by the antioxidant ionol, whose protective action is in all probability linked with its ability to inhibit activation of lipid peroxidation, caused by isoprenaline (unpublished data).

It will be evident that the mechanisms of tho disturbance of electrical stability of the heart in β-adrenergic injuries require further study. Meanwhile the data so far obtained suggest that stress-induced lesions, adrenergic in nature, of the myocardium, while not causing disturbances of cardiac contractility, nevertheless may be the cause of certain types of arrhythmias observed in clinical practice, and may perhaps be the cause of sudden cardiac death.

LITERATURE CITED

1. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
2. F. Z. Meerson and L. M. Belkina, Patol. Fiziol Éksp. Biol., No. 6, 1 (1986).
3. F. Z. Meerson, L. M. Belkina, S. S. Dyusenov, et al., Karalologlya, No. 10, 28 (1985).
4. P. Ganguly, P. Bora, S. Seth, et al., Curr. Ther. Res. Clin. Exp., **31**, 56 (1982).
5. Y. Joseph, A. Jordan, and T. Balazs, Pharmacology, **44**, 239 (1984).
6. S. Kumaris and V. P. Menon, Indian J. Exp. Biol., **25**, 419 (1987).
7. B. Lown, Circulation, **76**, No. 1, Suppl. Part 1, 186 (1987).
8. B. Lown, R. Desilva, P. Reich, et al., Am. J. Psychiat., **137**, 1325 (1980).
9. B. Lown, R. Verrier, and S. Rabinowitz, Am. J. Cardiol., **39**, 890 (1977).
10. F. Z. Meerson, V. V. Didenko, L. M. Belkina, et al., Cellular Antioxidant Defense Mechanisms [in Russian] (1988), pp. 215-245.
11. G. Rona, M. Boutet, J. Huttner, et al., Recent Advances in Studies on Cardiac Structure and Metabolism, Vol. 3, Baltimore (1973), pp. 507-514.
12. J. E. Skinner, J. Am. Col. Cardiol., **5**, No. 6, Suppl. 88 (1985).