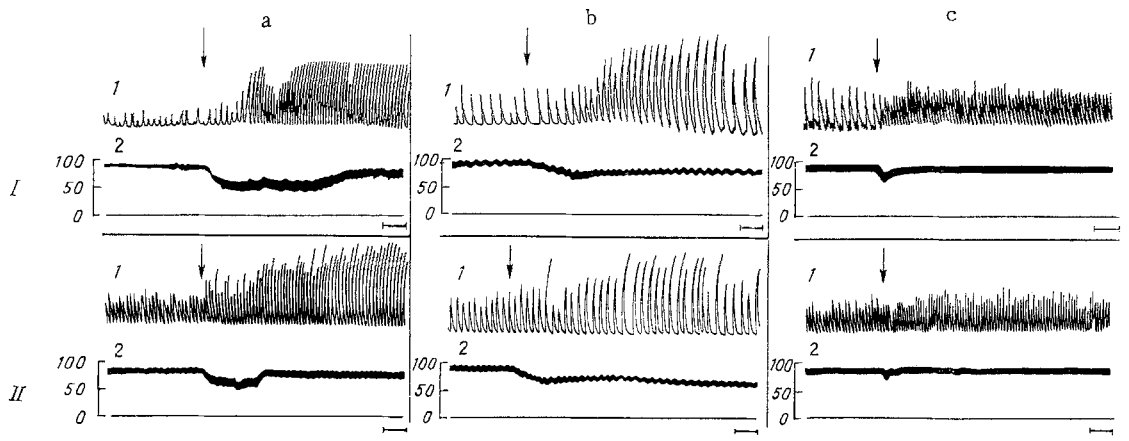

Fig. 1. Effect of naloxone (a), bradykinin (b), and TSHRF (c) on respiratory depression induced by morphine (I) and enkephalin analog (II). 1) Integrated EMG of diaphragm, 2) arterial pressure (in mm Hg). Arrow indicates time of injection of substance. Time marker 4 sec.

of respiration suggest that opioidergic reception in the respiratory system is heterogeneous. This situation can evidently explain differences in the action of naloxone, bradykinin, and TSHRF on respiration in the initial state and when inhibited by analgesics. The marked ability of bradykinin to increase mainly the depth, but of TSHRF to increase mainly the frequency of respiration suggests that their naloxone-like effect is mediated through different populations of opiate receptors participating in the regulation of breathing.

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USE OF THE ANTIOXIDANT IONOL TO PREVENT DAMAGE TO THE HEART CAUSED BY PROLONGED ANTITUBERCULOSIS MEDICATION

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UDC 615.281:579.873.21/.065:616.12/.015.25:615.243.4

KEY WORDS: isoniazid; prothionamide; rifampicin; heart; antioxidant ionol.

It was shown previously that prolonged administration of antibacterial antituberculosis preparations (ABP) to intact animals has a cardiotoxic action, manifested by marked destructive and dystrophic changes in the heart muscle [7]. A comparative study of different combinations of ABP showed that a combination of ABP such as isoniazid, prothionamide, and rifampicin may have a marked toxic action on the cardiovascular system [7].

The investigation described below had two aims: first, to detect and evaluate quantitatively functional and metabolic disturbances of the myocardium arising during prolonged administration of ABP, and second, to attempt to prevent these disturbances by means of the synthetic antioxidant ionol [1].

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 59-62, October, 1983. Original article submitted December 31, 1982.

 0007-4888/83/9610-1410$07.50

TABLE 1. Disturbances of Myocardial Contractility Caused by Prolonged Administration of Antituberculosis Drugs and Their Prevention by Antioxidant Ionol (M ± m)

Parameter	Group of animals	Heart rate, beats/min		
		120	300	400
Systolic pressure, mm Hg	1	84±2,9	98±3,0	122±5,1
	2	75±4,3	104±8,8	101±10,4
	3	51±7,9	56±8,8	55±8,1
	4	72±6,6	81±5,1	76±4,6
	5	90±3,1	96±2,9	95±4,5
	P_{1-2}	>0,05	>0,05	>0,05
	P_{1-3}	<0,02	<0,001	<0,001
	P_{1-5}	>0,05	>0,05	<0,001
	P_{4-5}	<0,05	<0,02	<0,02
Diastolic pressure, mm Hg	1	4,3±0,40	0,8±0,10	6,9±0,53
	2	6,0±0,77	4,2±1,13	9,8±2,60
	3	6,4±0,20	7,3±2,07	16,6±1,90
	4	6,4±0,83	7,3±2,29	17,8±4,60
	5	4,7±0,25	2,4±0,54	13,4±1,58
	P_{1-2}	>0,05	<0,01	>0,05
	P_{1-3}	<0,05	<0,01	<0,001
	P_{1-5}	>0,05	<0,02	<0,05
	P_{4-5}	<0,05	>0,05	>0,05
Velocity of contraction, mm Hg/sec	1	1341±87		
	2	1067±86		
	3	552±185		
	4	1195±286		
	5	2277±130		
	P_{1-2}	>0,05		
	P_{1-3}	<0,01		
	P_{1-5}	<0,001		
	P_{4-5}	<0,02		
Velocity of relaxation, mm Hg/sec	1	807±47		
	2	687±101		
	3	331±103		
	4	573±140		
	5	1052±81		
	P_{1-2}	<0,05		
	P_{1-3}	<0,001		
	P_{1-5}	<0,05		
	P_{4-5}	<0,02		
Diastolic defect, mm Hg/sec	1			26,5±2,3
	2			13,6±1,7
	3		18,7±15,0	42,7±6,1
	4		16,8±8,6	45,7±2,3
	5			29,5±6,8
	P_{1-2}			<0,05
	P_{1-3}			<0,05
	P_{1-5}			>0,05
	P_{4-5}			<0,05

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 190-230 g anesthetized with ether. The animals were divided into five groups: 1) control animals; 2) animals infected with *Mycobacterium tuberculosis*; 3) animals receiving isoniazid (15 mg/kg), prothionamide (25 mg/kg), and rifampicin (15 mg/kg) daily, perorally, for 2 months; 4) animals 1 month after discontinuation of ABP, and 5) animals receiving ionol (40 mg/kg) simultaneously with ABP in the same doses for 2 months. From seven to 12 animals were used in each series. Myocardial contractility was studied on the isolated heart, contracting isometrically at different frequencies (120, 200, 300, and 400 min^{-1}) around a latex balloon, filling the chamber of the left ventricle [10]. The heart was perfused with Krebs–Henseleit solution saturated with carbogen. The pressure in the left ventricle was measured by an electromanometer and recorded together with its first derivative, after which the systolic, diastolic, and developed pressure and also the velocity of contraction and relaxation and the diastolic defect were calculated [6]. Activity of creatine phosphokinase [2] and aspartate aminotransferase

[14] was determined in perfusate after flowing through the coronary system and the loss of these enzymes was calculated per gram dry weight of myocardium per hour.

It is essential to note that these experiments were done on the isolated heart under conditions when the principal determinants of cardiac activity were monitored; disturbances of the contractile function could therefore depend only on reasonably lasting damage to structure and metabolism arising through infection with tuberculosis and (or) prolonged administration of ABP. Lipids were extracted from the myocardium by the method in [11]. Accumulation of hydroperoxides in polyene lipids was estimated from the UV absorption spectra of solutions of lipids in a methanol–hexane (5:1) mixture, determined on the SF-26 spectrofluorometer [4], and end-products of lipid peroxidation (LPO), namely Schiff bases, were determined from fluorescence of the lipid extract in chloroform [9] on a BIAN-130 fluorometer, calibrated with a 1% solution of quinine sulfate in 0.1 N H_2SO_4. Concentrations of ATP, ADP, and AMP were determined by the "Test-Combination" kit from Boehringer Mannheim (West Germany).

EXPERIMENTAL RESULTS

If the initial frequency was 120 contractions per minute depression of the contractile function was observed and was most marked in the group of animals receiving prolonged ABP medication, in which the systolic pressure was reduced by 1.6 times and the velocity of contraction and relaxation by 2.4 times (Table 1). The myocardial contractility was improved 1 month after discontinuing ABP. Infection of the animals with tuberculosis did not cause depression of myocardial contractile function and parameters such as velocity of contraction and relaxation were actually higher than in the control.

When a higher frequency of contractions was imposed the cardiotoxic effect of ABP was even more clearly marked: Whereas the heart of the control rats responded to an increase in the frequency of contraction with a rise of developed pressure by 45% (positive inotropic effect), the heart of rats receiving ABP responded to an increasing frequency of contractions by a reduction in developed pressure by 18% (negative inotropic effect) and by disturbance of diastolic relaxation of the myocardium. This was expressed quantitatively as an increase of 2.6 times in the diastolic pressure (1.5 times in the control) but the diastolic defect now appeared at a frequency of 300 beats/min and was almost twice as high as the control level at a frequency of 400 beats/min, evidence of reduced efficiency of work of the membrane calcium pump of the myocytes, which is responsible for the timely and sufficiently complete removal of Ca^{++} from the myofibrils and realization of diastolic relaxation.

TABLE 2. Effect of Prolonged Administration of ABP on Activation of LPO and Energy Metabolism of Rat Myocardium (M ± m)

Parameter	Group of animals		
	1	3	5
Concentration of lipid hydroperoxides, nmoles/mg lipids	5,1±0,33	11,7±1,02 $P_{1-2}<0,001$	6,9±0,64 $P_{1-3}<0,05$ $P_{3-5}<0,001$
Intensity of fluorescence of Schiff bases, relative units	3,95±0,50	10,70±1,57 $P_{1-2}<0,001$	6,70±0,97 $P_{1-3}<0,05$ $P_{3-5}<0,05$
ATP, μmoles/g	2,01±0,096	2,00±0,190 $P_{1-2}>0,05$	2,45±0,130 $P_{1-3}<0,05$ $P_{2-3}>0,05$
ADP, μmoles/g	0,41±0,023	1,130±0,070 $P_{1-2}<0,001$	0,96±0,057 $P_{1-3}<0,001$ $P_{2-3}>0,05$
AMP, μmoles/g	0,14±0,006	0,19±0,047 $P_{1-2}>0,05$	0,14±0,030 $P_{1-3}>0,05$ $P_{2-3}>0,05$
Energy potential of cell	0,862±0,027	0,774±0,080 $P_{1-2}>0,05$	0,825±0,060 $P_{1-3}>0,05$ $P_{2-3}>0,05$

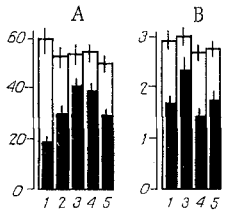

Fig. 1. Release of aspartate aminotransferase (A) and creatine phosphokinase (B) into coronary blood flow. Abscissa, group of animals; ordinate, enzyme activity. Activity of enzymes expressed in μmoles of products, i.e., pyruvic acid and creatine, formed per hour at 37°C after addition of 1 ml of coronary perfusate to incubation medium, and calculated per gram dry weight of tissue per hour. Unshaded columns – enzyme activity 5 min after beginning of perfusion of hearts, black columns – the same, 30 min after beginning of perfusion.

The possible causes of depression of myocardial contractility caused by prolonged administration of ABP were either a deficiency of high-energy phosphorus compounds or a disturbance of membrane transport of Ca^{++}. As the results show, the ATP level in the myocardium of rats receiving ABP was indistinguishable from the control, the ADP level was 2.7 times higher, and the AMP level 1.4 times higher (Table 2). This was accompanied by a decrease in the ATP/ADP ratio from 4.90 to 1.77 and it indicated an increase in ATP turnover. The energy potential of the cell, calculated by Atkinson's method [8], showed a tendency to fall by 18%. Consequently, depression of myocardial contractility could hardly be explained by ATP deficiency. The most likely cause of disturbances of myocardial contractility in animals receiving ABP was injury to the membrane Ca^{++} transport mechanisms in the cardiomyocytes by these drugs. This hypothesis was confirmed by at least three facts. First, prolonged administration of ABP is accompanied by excessive activation of LPO, with an increase in concentration of hydroperoxides in lipids isolated from heart muscle by 2.3 times, and an increase in the content of Schiff bases by 2.7 times (Table 2). The damaging effect of LPO in biomembranes consists essentially of a change in the lipid microenvironment of membrane-bound enzymes and ion-conducting channels, the formation of new permeability channels, oxidation of SH groups in active centers of membrane-bound enzymes, and the formation of cross-linkages between proteins and phospholipids of biomembranes, with their fragmentation and destruction [3, 5, 12, 13]. Second, whereas 5 min after the beginning of perfusion the release of enzymes into the coronary blood flow was quite high (Fig. 1) and did not depend on the experimental conditions, which is evidently the result of such harmful factors as "sacrifice stress" and "dissection hypoxia" [4], after 30 min the cardiotoxic effect of ABP was already manifested quite clearly. The hearts of rats receiving ABP for 2 months evidently secrete into the coronary blood flow 1.4 times more creatine phosphokinase and 2.0 times more aspartate aminotransferase than in the control, and 1.5 times more than the hearts of animals infected with tuberculosis. The release of these enzymes correlates closely with disturbances of myocardial contractility, reflecting the degree of damage to the cardiomyocyte membranes. Third, simultaneous injection of ABP and the antioxidant ionol, which is an effective "trap" for peroxide radicals [1], considerably reduced the content of lipid hydroperoxides and Schiff bases in the myocardium, prevented disturbance of its contractile function, and reduced enzyme release into the coronary blood flow; it also improved the energy metabolism of the myocardium by increasing the ATP concentration and the energy potential.

Excessive activation of LPO in lipids of myocyte membranes, caused by prolonged administration of ABP, evidently plays the role of a trigger mechanism in the pathogenesis of their cardiotoxic action, and the use of ionol in the treatment of tuberculosis merits serious clinical and physiological study.

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EFFECT OF PRELIMINARY ADMINISTRATION OF THE α-ADRENOBLOCKER PHENTOLAMINE AND THE β-ADRENOBLOCKER INDERAL ON STRESS-INDUCED FALL OF PORTAL VEIN RESPONSE TO NORADRENALIN

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UDC 616.149-009.1-02:
613.863/-02:615.217.24

KEY WORDS: stress; α-adrenoblocker phentolamine; β-adrenoblocker inderal.

Profound depression of spontaneous contractile activity of the smooth muscle of the portal vein and a sharp fall in its adrenoreactivity are known to occur as a result of previous severe emotional-painful stress (EPS), and they may play an important role in the pathogenesis of arterial hypovolemia and of states resembling collapse [2, 4]. At the same time, it has been shown that stress-induced damage to heart muscle can be effectively prevented by means of the β-adrenoblocker inderal [3, 5].

Since the adrenoreceptor apparatus in the smooth muscles of blood vessels consists of two types of receptors [6], the aim of this investigation was to study the effect of preliminary administration of the α-adrenoblocker phentolamine and the β-adrenoblocker inderal on contractility and adrenoreactivity of the isolated portal vein after EPS.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-220 g. Six groups of rats were used: 1) control, 2) control receiving inderal, 3) control receiving phentolamine, 4) exposed to EPS, 5) receiving inderal before the beginning of exposure to EPS, 6) receiving phentolamine before the beginning of EPS.

The α-adrenoblocker phentolamine and the β-adrenoblocker inderal were injected intraperitoneally in doses of 5 and 1 mg/kg respectively, 1 h before the beginning of exposure to stress.

EPS was produced by Desiderato's method [7] in the course of 6 h. The animals were decapitated 2 h after the end of exposure to stress and the portal veins were removed and placed in thermostatically controlled working chambers perfused with oxygenated Krebs' solution at a temperature of 32°C and with a load of 400 mg [4]. Spontaneous contractile activity was recorded on a two-channel system (Ugo Basile, Italy).

The following parameters of contractile activity were calculated: the developed tension, the frequency of phasic contractions per minute, the intensity of functioning of structures (IFS), equal to the product of the developed tension and frequency of contractions, calcula-

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 0007-4888/83/9610-1414$07.50