EFFECT OF CLINICAL DEATH AND PRELIMINARY INJECTION OF PRACTOLOL ON ADRENORECEPTOR SENSITIVITY OF THE RAT HEART IN THE POSTRESUSCITATION PERIOD

V. T. Dolgikh

UDC 616.12-008.315-02:615.217.24/-092.9-07

KEY WORDS: heart; clinical death; resuscitation; practolol.

Activation of the sympathicoadrenal system during the terminal state and in the early period after resuscitation is responsible for hyperdynamia of the myocardium during the first few minutes after resumption of cardiac contractions [2]. Meanwhile, prolonged hypercatecholaminemia may have a damaging action on the heart muscle due to excessive stimulation of β-adrenoreceptors by high concentrations of catecholamines [11, 12]. If this view is correct, preliminary (before clinical death) adrenoreceptor blockade by the nonselective β-blocker practolol ought to significantly reduce postresuscitation heart damage.

In the investigation described below the effect of preliminary practolol on adrenoreceptor sensitivity of the rat heart was determined in the postresuscitation period.

EXPERIMENTAL METHOD

Experiments were carried out on 170 noninbred male rats weighing 180-210 g, anesthetized by intraperitoneal injection of pentobarbital (25 mg/kg). There were four series of experiments: 20 animals constituted the control (series I), 106 rats were subjected to clinical death (series II), 20 rats received practolol (series III), and 24 rats received practolol before clinical death (IV). Clinical death lasting 4 min was induced by bleeding from the carotid artery, and resuscitation took the form of centripetal injection of the previously withdrawn blood, accompanied by artificial ventilation of the lungs and indirect cardiac message. Preparations stimulating the cardiovascular system were not used on two counts: first, in the resuscitation experiments on small laboratory animals, after acute blood loss, mechanical asphyxia, and acute total ischemia, produced by compression of the neurovascular bundle at the base of the heart, adrenalin is not used [1, 4, 5, 8, 10], so that the results of the present series of experiments could be compared with greater reliability with data in the literature, and second, in the investigation described below the degree of cardiac damage and adrenoreceptor sensitivity of the heart were studied specifically in the postresuscitation period, and for that reason drugs (especially catecholamines) stimulating the cardiovascular system, and which could cause additional damage to the heart muscle, were excluded. The heart was removed 1.5 h after resuscitation, when signs of marked heart failure were noted, in order to study adrenoreceptor sensitivity to noradrenalin (NA). For this purpose, the hearts were placed in Krebs–Henseleit solution, cooled to 4°C, the right atrium was removed, and the atrial septum was ligated, in order to create an atrioventricular block. A small latex balloon of constant volume, on compression of which the ventricle performed isometric contractions with a frequency of 120 min^{-1}, imposed by means of an ÉSU-2 electrical stimulator, was introduced into the left ventricle. The heart was perfused with the same solution, saturated with carbogen (95% O_2 + 5% CO_2), under a pressure of 70 mm Hg at 37°C and at pH 7.34-7.36. The pressure in the left ventricle was measured by means of a BMT electromanometer (East Germany) and recorded on an N338-4P instrument. After perfusion for 30 min, when the work of the heart had stabilized, its adrenoreceptor sensitivity was estimated from the systolic pressure developed by the left ventricle in response to addition of increasing doses of NA to the perfusion fluid ($5 \cdot 10^{-8}$; $1 \cdot 10^{-7}$; $2.5 \cdot 10^{-7}$; $5 \cdot 10^{-7}$; $1 \cdot 10^{-6}$, and $2 \cdot 10^{-6}$ μg),

Department of Pathophysiology, Central Research Laboratory, Omsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR E. D. Gol'dberg.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 9, pp. 257-259, September, 1990. Original article submitted October 8, 1989.

 0007-4888/90/0009-1190$12.50

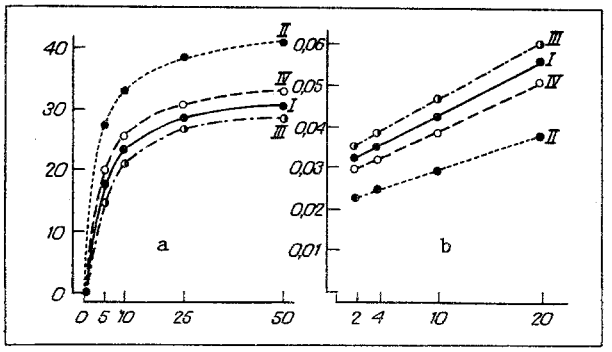

Fig. 1. Adrenoreceptor sensitivity of rat heart in postresuscitation period. I-IV) Series of experiments. a) Curve showing effect of NA on ionotropic reaction of isolated heart. Abscissa, quantity of NA ($\times 10^{-8}$, in μg) injected into perfusion system; ordinate, rise of systolic pressure (in % of initial value taken as 100%); b) dependence of inotropic response of isolated heart on NA concentration. Abscissa, reciprocal of amount of NA injected into perfusion system (in μg, $1/10^6$); ordinate, reciprocal of inotropic effect (1%).

TABLE 1. Effect of Preliminary Administration of Practolol on Adrenoreceptor Sensitivity of the Heart to Noradrenalin ($M \pm m$)

Series of experiments	Number of observations	Apparent dissociation constant of NA–adrenoreceptor complex, µg
Control (I)	10	$(13,7\pm1,42)\cdot10^{-8}$
Resuscidation (II)	12	$(6,9\pm0,90)\cdot10^{-8*}$
Practolol (III)	10	$(16,4\pm1,74)\cdot10^{-8}$
Practolol + resuscitation (IV)	12	$(11,3\pm0,77)\cdot10^{-8*}$

Legend. Here and in Table 2 asterisks indicate significant differences between experiments of series I and II, and III and IV.

after which graphs were plotted to show how the effect of NA depends on its concentration in the perfusion fluid, using a double Lineweaver–Burk system of reciprocal coordinates, by means of which the apparent dissociation constants (K) of the NA – adrenoreceptor complex could be calculated. The value of K was numerical equal to the concentration of NA inducing a response equal to half of the maximal value [6]. During the terminal state and the 30-min recovery period the ECG was recorded continuously in lead II. At various times after resuscitation the NA and adrenalin (A) concentrations in the heart muscle were determined by the trihydroxyindole method. Practolol (Inderal, from ICI, England) was injected intraperitoneally in a single dose of 1 mg/kg (30 min before bleeding). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The sensitivity of the isolated rat heart removed 1.5 h after resuscitation to NA is illustrated in Fig. 1. Clearly the same dose of NA, injected into the perfusion system, caused an increase of systolic pressure by 43 ± 1.5%, compared with 26 ± 1.7% in the control. This method of assessing the action of a substance by the ratio of the effect after to the effect before [6] has the essential disadvantage that it cannot be used to judge whether the value obtained is the greatest possible value. The possibility cannot be ruled out that in the presence of high concentrations of NA its cardiotoxic action may be manifested and, as a result, the magnitude of the response may be reduced. By plotting a graph showing dependence of developed pressure on NA concentration in the perfusion fluid, in a system of double reciprocal coordinates, and using the graph to determine K for the NA – adrenoreceptor complex, it is possible to avoid mistakes connected with the evaluation of the adrenoreceptor sensitivity of the heart.

TABLE 2. Effect of Preliminary Administration of Practolol on Postresuscitation Changes in Catecholamine Concentrations in Rat Heart ($M \pm m$)

Series of experiments	Number of observations	NA, mg/g	A, mg/g
Control (I)	10	1322±42	56±6
Resuscitation, 5 min	12	860±32*+	94±12
» 30 min	11	731±40*+	98±13*+
» 90 min (II)	12	905±54*+	72±9
Practolol (III)	10	1395±163	60±10
Practolol + resuscitation (IV)	10	1178±96*	53±11*

Legend. Plus signs indicate significant differences from control.

As will be clear from Fig. 1 and Table 1, K for the NA — adrenoreceptor complex was reduced by half 1.5 h after resuscitation, evidence of increased sensitivity of the heart to exogenous NA, which was evidently associated with a decrease in its level in the heart muscle. This conclusion is confirmed by the data in Table 2. Clinical death and resuscitation reduced the NA concentration in the myocardium at different times by 35-45% and increased the A concentration by 29-75%, evidently through its uptake by cardiomyocytes from the circulating blood.

An increase in the concentration of A, which has an arrhythmogenic effect [9, 11, 12], in the myocardium regularly caused rhythm disturbances in the form of single and grouped extrasystoles, which appeared at one or more foci of excitation. In 14.6% of cases the extrasystoles terminated in ventricular fibrillation and death of the animals. The twofold increase in sensitivity of the adrenoreceptors of the heart to catecholamines and the excess of their level in the blood in the early period after resuscitation [7] were evidently the key factor in the pathogenesis of postresuscitation heart failure and functional-metabolic and structural damage to the heart described by the writers previously [2].

β-Adrenoreceptor blockade in the intact animals revealed no significant differences in the test parameters compared with the control (Fig. 1; Tables 1 and 2). Meanwhile, injection of practolol 30 min before clinical death prevented ventricular fibrillation and reduced the number of extrasystoles by several times in the first half-hour after resuscitation (from 59 ± 8.0 to 6 ± 2.2). Ultimately the drug reduced mortality in the first 1.5 h after resuscitation from 55.1 to 7.7%.

Preliminary β-adrenoreceptor blockade in the heart by practolol prevented accumulation of A and reduction of NA in the heart of animals surviving clinical death: the absolute values of these parameters did not differ significantly from the control level. This pattern was regularly accompanied by a decrease in sensitivity of the β-adrenoreceptors of the heart to exogenous NA (the Cannon—Rosenblueth principle). It will be clear from Table 1 that K of the NA — adrenoreceptor complex of the heart of animals protected with practolol was almost doubled form (6.9 ± 0.90) × 10^{-8} to (11.3 ± 0.77) × 10^{-8} μg NA and it did not differ significantly from the control.

Thus β-adrenoreceptor blockade before clinical death by practolol, by limiting excessive stimulation of the adrenoreceptors by catecholamines, restores the normal catecholamine concentrations in the heart muscle and sensitivity of the heart muscle to exogenous NA; as we showed previously [2, 3], this significantly reduces the severity of postresuscitation damage to the heart.

LITERATURE CITED

1. N. A. Baranets, V. T. Dolgikh, and R. P. Nartsissov, Byull. Éksp. Biol. Med., No. 10, 405 (1985).
2. V. T. Dolgikh, "Damage to and protection of the heart in acute lethal blood loss," Author's Abstract of Doctoral Dissertation, Medical Sciences, Tomsk (1987).
3. V. T. Dolgikh and F. Z. Meerson, Anest. Reanimatol., No. 1, 10 (1989).
4. A. S. Zinov'ev, P. G. Mal'kova, A. M. Kochetov, and V. A. Rezina, Arkh. Patol., No. 3, 54 (1988).
5. V. G. Korpachev, S. P. Lysenkov, and L. Z. Tel', Patol. Fiziol., No. 3, 78 (1982).
6. B. N. Manukhin, Physiology of Adrenoreceptors [in Russian], Moscow (1968).
7. V. A. Negovskii, A. M. Gurvich, and E. S. Zolotokrylina, Postresuscitation Sickness [in Russian], Moscow (1987).
8. Yu. V. Red'kin, T. F. Sokolova, and A. A. Zubakhin, Patol. Fiziol., No. 2, 36 (1988).
9. D. B. Saprygin, R. A. Serov, L. S. Kashtelyan, et al., Byull. Éksp. Biol. Med., No. 8, 175 (1984).
10. S. S. Stepanov, G. E. Gaponenko, and V. V. Semchenko, Patol. Fiziol., No. 6, 79 (1983).

11. L. H. Opie, Catecholamines and the Heart: Recent Advances in Experimental and Clinical Research, Berlin (1981).
12. A. M. Wheatley, F. T. Thandroyen, and L. H. Opie, J. Molec. Cell. Cardiol., 17, No. 4, 349 (1985).

NEUTRALIZATION OF THE TOXIC ACTION OF ENDOTOXINS OF GRAM-NEGATIVE BACTERIA BY UNITHIOL AND MAGNESIUM SULFATE

M. I. Grutman, Yu. V. Persidskii, V. M. Frolov, R. N. Nagornaya-Persidskaya, and E. P. Gushla

UDC 615.919:579.842].015.2:615.244.9].07

KEY WORDS: endotoxins; lipid peroxidation; liver; lungs; unithiol

The mechanism of action of endotoxins on the living organism is very complex and has not been adequately studied. One of the results of this action is the formation of free oxygen and hydroxyl radicals and, as a result of this, intensification of lipid peroxidation (LPO), destabilization of the cell membranes, swelling of mitochondria, and so on [1, 5, 8]. It can accordingly be postulated that antioxidants may partially neutralize the action of endotoxins and may prove to be effective agents for the treatment of infectious diseases caused by Gram-negative microorganisms. According to some reports, metallothionein proteins synthesized in the body and the particular feature of which is their high content of sulfhydryl groups and ability to bind ions of certain metals, play an important role in protection of the organism against endotoxins [7]. It was accordingly suggested that drugs carrying sulfhydryl groups and possessing antioxidative properties (in particular, unithiol), could prove to be effective agents protecting the body against the action of endotoxins. The protective action of unithiol and of a combination of unithiol with magnesium sulfate was studied in poisoning caused by breakdown products of *Salmonella typhimurium* and *Shiqella sonnei,* and on the cyclic adenosine monophosphate (cAMP) level during this type of poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 17-20 g. A lysate of the bacteria was prepared by ultrasonic irradiation of a suspension of a 24-h agar culture in the UZDN-1 apparatus (4 min, 44 kHz). The sonicated suspension was centrifuged (15 min, 35,000g), and the supernatant was sterilized by filtration through membrane filters.

LD_{50} of the lysate was determined by probit analysis, the readings being taken after 48 h [2]. The malonic dialdehyde (MDA) concentration was determined by the reaction with 2-thiobarbituric acid and the cAMP was determined with the aid of kits from Amersham International (England) [4]. The results were subjected to statistical analysis by Student's t-test [3].

The plan of the experiments was as follows. Mice were injected intraperitoneally with lysate of *S. Typhimurium* or *Sh. sonnei* in a volume of 0.2 ml. To determine MDA and cAMP, the dose of the lysate was equal to LD_{50}. To study the protective action of unithiol LD_{50} was determined for the experimental and control groups. For this purpose each group was divided into six subgroups each containing five mice. The dose was increased in steps of 1.5.

The mice of the experimental group began to receive unithiol immediately after injection of the lysate. The preparation was injected intramuscularly in a dose of 0.5 mg in a volume of 0.2 ml. Considering that the half-elimination time of unithiol is 2-3 h, the first two injections were given with an interval of 3.5 h. Later, the injections were given every 5 h. Magnesium sulfate was injected twice a day at intervals of 12 h, in a dose of 0.13 mg.

Research Institute of Epidemiology and Infectious Diseases, Ministry of Health of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 9, pp. 259-261, September, 1990. Original article submitted July 4, 1989.