Respiration Restitution in Rats Following Its Termination in Immersion Hypothermia

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Rats were cooled in water until attaining profound hypothermia and respiratory arrest. After removal from water, 0.5% solution of Na_2EDTA was administered intravenously. This led to a drop of blood $[Ca^{2+}]$ by 20-30% from the baseline and promoted recovery of respiration following its arrest lasting 10.3±1.4 min. By the 30th minute of Na_2EDTA administration, respiration rate increased to 32.3±5.2 cycles per minute and respiration amplitude reached 68±4% of the baseline level. This effect was observed without special warming of the rats. It was concluded that the period during which the organism maintains viability in respiration arrest and disturbances in respiratory center are still reversible is prolonged under conditions of profound hypothermia.

Key Words: *immersion hypothermia; respiration; calcium; Na_2EDTA*

Body temperature can significantly decrease during long-term stay of the human in cold water, which results in disturbances of vital physiological functions of the body and lethal outcome. The maintenance of pulmonary respiration is important for life sustaining in people with developed profound hypothermia. Intensive care usually includes artificial respiration and warming of the body. However, warming in profound hypothermia is fraught with adverse consequences, since the main body functions are inhibited and adequate energy supply of tissues and organs cannot be provided. The development of the most efficient methods of getting person out of profound hypothermia, based on the discovering of the mechanisms of cold function depression, is of fundamental importance. However, elimination of cold paralysis of physiological function in homoiothermic organism is far from complete solution.

The development of cold stress of nervous cells is based on the processes determined by inhibition of ATP synthesis, which results in disturbances of energy-dependent calcium ion transport out of the cell into the extracellular medium against high concentration gradient of this ion. It results in prolonged increase of $[Ca^{2+}]$ in cell cytoplasm above normal values, which in turn induces activation of phospholipases and Ca^{2+}-dependent proteases, dysfunction, and cell death [2,5,6]. For good reasons, elucidation of regularities of respiration inhibition and recovery in human in cases of profound accidental hypothermia is impossible; hence these questions should be solved in animal experiments. Our previous experiments showed that activation of thermoregulation and respiration functions in rats in profound hypothermia without warming can be obtained using intravenous injection of disodium salt of ethylenediaminotetraacetic acid, Na_2EDTA (in doses lowering blood concentration of calcium ions by 20-30%). The increase in respiration rate and amplitude were observed after Na_2EDTA administration to rats cooled in water to a rectal temperature of 17-15°C [1], as well as during cooling on air to a rectal temperature of 25-22°C [3,4]. It should be noted, that these effects were observed when breathing was infrequent, but not yet terminated.

The objective of this study was to investigate the possibility of using Na_2EDTA for respiration resump-

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tion after its prolonged arrest in rats under conditions of profound immersion hypothermia.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=25) weighing 310±15 g under Nembutal anesthesia (35-40 mg/kg intraperitoneally); the catheters were inserted into the femoral vein and artery and thermocouples were inserted into the brain. Heart rate was evaluated by *RR* interval on ECG recorded in standard lead II; respiratory rate (RR) and amplitude (RAmp) were recorded using a carbonic detector. Blood pressure was measured in the femoral artery (mercury manometer). EMG was registered: electrical activity of back muscles (thermoregulatory tonus, cold shivering) and biopotential area integral was estimated (S, μV×sec). Temperature in the rectum (tr) and in the area of medulla oblongata (tm) was measured using copper-constantan thermocouples and photoelectric amplifier F-116/2. After 2.5-3 h the rats in the special carriage were submersed in water bath (10.3±0.3°C); rat body was placed in the slope, head and upper back were situated above water surface. The animals were cooled until respiratory arrest and then were taken out of water. After 3-7 min (5.0±0.7 min), 0.5% Na_2EDTA solution in physiological solution at the rate of 4-5 drops per minute (0.00268-0.00335 mmol/min) was administered using the dropper into the femoral vein for 5 min. Second dose of Na_2EDTA was administered 15-22 min after the initial dose. Blood samples (0.3 ml in volume) were taken from the femoral vein before cooling, before first Na_2EDTA administration, and 8 min after the first and second administrations. $[Ca^{2+}]$ in blood samples was measured using calcium ion-selective electrodes. Blood samples for $[Ca^{2+}]$ estimation were taken from the control rats at the same terms.

Multichannel biopotential amplifier, 12-bit 8-channel analog-digital converter and hardware-software instruments for physiological experiment automatization were used for registration and saving of the data.

Statistical treatment of the obtained results was carried out using Statistica 6.0 software. Mean values (M) and error of the mean (m) were calculated, t test was used for dependent variables (P_w).

RESULTS

Baseline tr in rats was 36.8±0.5°C, tm 36.2±0.6°C, RR 97±5 min^{-1}, HR 478±11 bpm, and BP 96±5 mm Hg. Baseline RAmp was taken for 100%. The rate of tr drop during the first 10 min after submersion reached 0.72±0.04°C/min and tm 0.62±0.07°C/min; by the 50-60th minutes the rate of parameter drop decreased to 0.15±0.02°C/min. Mean time of rat cooling was 66±5 min. Cold shivering was maximum at tr 25-28°C and then gradually decreased and disappeared at tr 16.3±0.3°C and tm 17.7±0.5°C. In most rats, arrhythmia and HR fluctuations were recorded at tr 15.0±0.2°C and tm 16.1±0.3°C. The rats were taken out of water when breathing stopped or 1-2 breathing movements were observed in one minute (gasping). At the next stage of the experiment, the rats stayed at room temperature 17.8±0.5°C, however, tr and tm further decreased by 0.8-1.1°C over 13.6±1.2 min (afterdrop effect).

In the experimental group (n=17), 0.5% Na_2EDTA solution was administered intravenously 5.0±0.7 min after taking out of water against the background of respiratory arrest. Restoration of breathing was observed in 17 rats, individual differences in the responses were noted. Thus, latency of the reaction was 2-4 min in 8 animals, 6-7 min in 4 animals, and 13-16 min in 5 animals. The duration of respiratory arrest in different rats varied from 5 to 18 min (10.3±1.4 min).

TABLE 1. Changes in the Studied Parameters in Hypothermic Rats before and after Intravenous Administration of 0.5% Na_2EDTA (n=12)

Stage of experiment	$[Ca^{2+}]$, mM	tr, °C	tm, °C	RR, min^{-1}	HR, bpm	BP, mm Hg
Before first Na_2EDTA administration	1.13±0.01[1]	15.1±0.3	16.4±0.4	0	53.5±4.2	42±5
8th minute following first Na_2EDTA administration	0.81±0.02[2]	14.6±0.3	15.7±0.4	6.4±1.5	51.4±3.8	46±5
8th minute after second (30th minute after the first) Na_2EDTA administration	0.72±0.02[3]	14.7±0.4	15.8±0.5	32.3±5.2	59.1±5.6	48±7
1 h after rats were taken out from water	—	15.8±0.6	16.4±0.5	39.7±5.3	68.9±3.5	49±6
2 h	—	16.6±0.3	17.3±0.4	42.8±4.4	81.5±2.6	50±4
3 h	0.98±0.03	17.1±0.4	17.7±0.5	51.7±5.2	87.4±3.7	51±5

Note. $Pw_{1,2;\ 1,3}<0.01$.

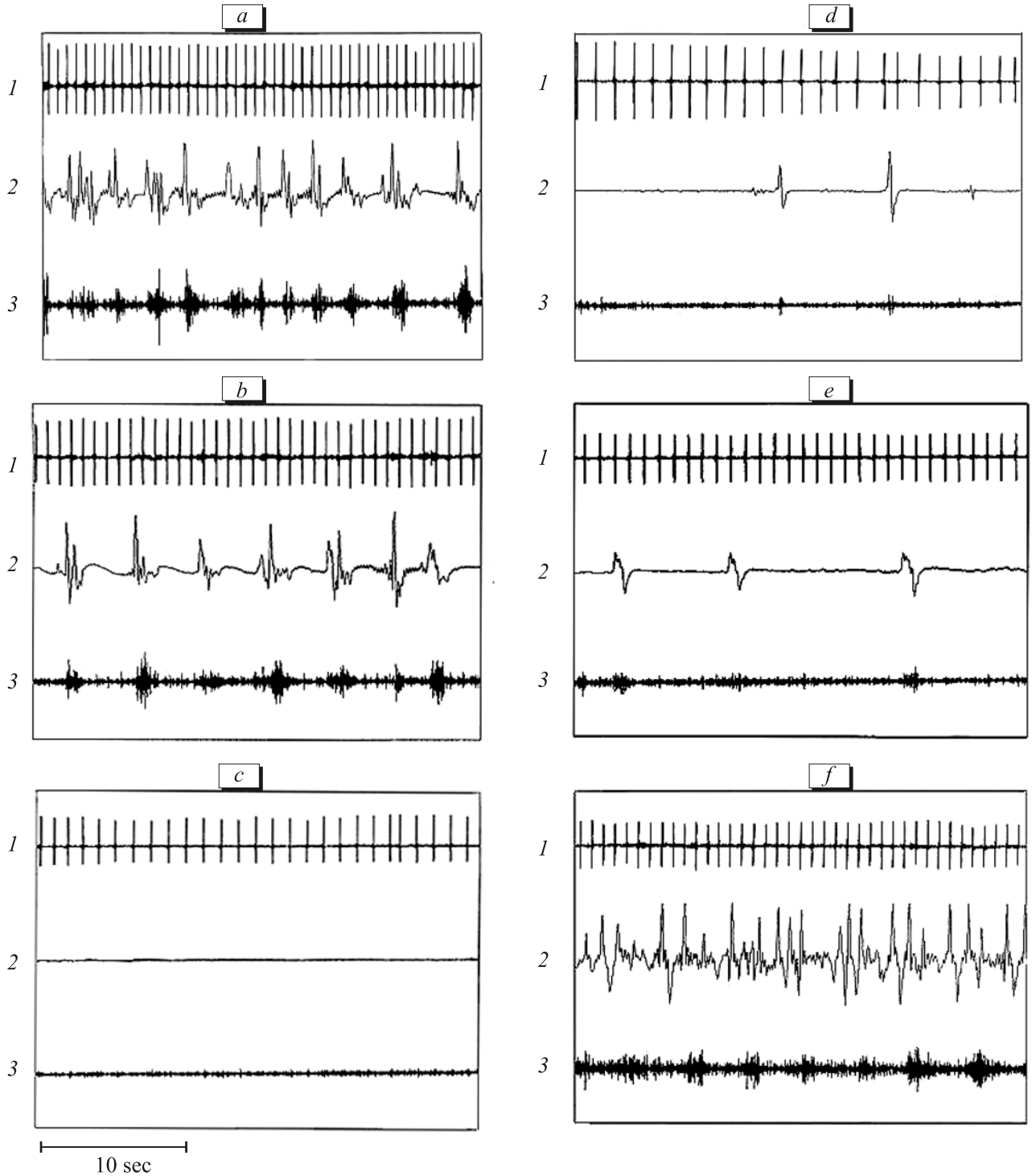

Fig. 1. Changes in HR (*1*), RR (*2*) and muscle electrical activity (*3*) during cooling in water (*a*, *b*) and after taking out from water (*c*-*f*) in one of experiments. *a*: tr 16.2°C, tm 17.8°C. *1*) 93 bpm, *2*) 41 min^{-1}, *3*) 1412 arb. unit/min; *b*: tr 15.4°C, tm 16.5°C. *1*) 72 bpm, *2*) 23 min^{-1}, *3*) 1126 arb. unit/min; *c*: 3 min after rat was taken out from water (1 min before the first Na_2EDTA administration). tr 14.8°C, tm 16.2°C. *1*) 51 bpm, *2*) 0 min^{-1}, *3*) 468 arb. unit/min; *d*: 8 min after the start of first Na_2EDTA administration. tr 14.6°C, tm 15.8°C. *1*) 47 bpm, *2*) 6 min^{-1}, *3*) 550 arb. unit/min; *e*: 22 min after onset of the first Na_2EDTA administration. tr 14.7°C, tm 15.6°C. *1*) 62 bpm, *2*) 7 min^{-1}, *3*) 685 arb. unit/min; *f*: 30 min after the start of the first Na_2EDTA administration (8 min after the second administration). tr 15.0, tm 15.8°C. *1*) 78 bpm, *2*) 47 min^{-1}, *3*) 1520 arb. unit/min.

The animals were divided into 2 groups according to the intensity of respiration activation. In 5 rats, the reaction was transient: RR increased from 0 to 8-14 min^{-1}, RAmp increased to 50.0±4.5% on minutes 4-8 after the start of Na_2EDTA administration. Respiration activation lasted for about 15 min, thereafter breathing stopped.

TABLE 2. Comparison of Physiological Parameters, Registered during Termination of Cold Shivering and Respiration in Hypothermic Rats and during Restitution of These Functions following Intravenous 0.5% Na_2EDTA Administration (n=12)

Parameter	Shivering termination	Taken out from water	Respiration termination	Respiration restitution	Shivering restitution
tr, °C	16.3±0.3[1]	15.3±0.4	15.2±0.3[2]	14.5±0.2[3]	15.3±0.4[4]
tm, °C	17.7±0.5[5]	16.5±0.4	16.6±0.3[6]	15.8±0.3[7]	16.2±0.6[8]
RR, min^{-1}	19.5±3.4	1.0±0.5	0	4.3±0.8	20.2±3.1
HR, bpm	78.3±4.9	62.6±5.2	55.1±6.2	45.1±3.8	62.4±4.3
BP, mm Hg	80.0±5.1	61.0±9.2	50.1±7.0	47.7±6.4	49.5±8.6

Note. $Pw_{1,4;\ 2,3;\ 5,8;\ 6,7}<0.001$; $Pw_{5,8;\ 6,7}<0.01$.

In 12 rats, the stimulating response to Na_2EDTA administration was pronounced (Fig. 1, Table 1). Initially, infrequent respiratory movements with RAmp 14.3±1.5% appeared, thereafter their rate and frequency gradually increased and by minute 30 after the first Na_2EDTA administration, the mean HR was 32.3±5.2 bpm and RAmp increased to 68±4%. The animals from this group exhibited bursts of cold shivering following respiration restitution. tr and tm at the moments of respiration restitution and cold shivering were lower than at the moment of their termination (Table 2). No relationships were discovered between the latency of the response and its intensity: more rapid RR increase could be observed in rats with longer latency of the response to Na_2EDTA administration, whereas in cases of rapid response maximal RR value sometimes did not exceed 15-20 min^{-1}.

Control rats (n=8) received no Na_2EDTA. Respiration stopped at tr 15.7±0.3°C (tm 16.7±0.3°C) in these animals and no respiratory movements were observed after removal from water.

During rat cooling to tr 17°C, BP did not decline below 81.2±5.3 mm Hg, at tr 15°C it decreased to 49.7±5.2 mm Hg. At tr 15-14°C the majority of animals exhibited BP fluctuations within 40-20 mm Hg, sometimes BP dropped to 0. Survivors demonstrated such fluctuations for 15 min, BP stabilized after respiration restitution, for 3 h it remained at 40-50 mm Hg (Table 1).

In controls, BP fluctuated within the range of 30-10 mm Hg for 15-20 min after respiration arrest and dropped to 0 mm Hg. ECG was recorded for 36±9 min after respiration arrest.

Before cooling, $[Ca^{2+}]$ concentration in all rats was 0.98±0.02 mM. In rats from the experimental group, it slightly increased before the first Na_2EDTA administration, but 8 min after the first and second administrations it decreased by 20-30% from the baseline level (Table 1). In control animals, $[Ca^{2+}]$ concentration after removal from water was 1.22±0.03 mM, by the 8th minute it was 1.19±0.02 mM, and by the 20-30th minute 1.27±0.03 mM.

Thus, experimental data led us to a conclusion that the decrease in blood $[Ca^{2+}]$ in hypothermic rats by 20-30% from normal values by Na_2EDTA administration can promote respiration restitution even after its complete arrest. One may assume that individual differences in the latency of responses to Na_2EDTA and maximum RR values observed during respiration resumption in rats were determined by different severity of cold stress in central structures of respiration regulation. It can be concluded that the period during which the organism retains survivability in respiration arrest and disturbances in respiratory center are reversible is prolonged under conditions of profound hypothermia

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