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Effect of Lithium Hydroxybutyrate on Hemodynamics and Respiration in Rats with Different Resistance to Hypoxia

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Acute experiments on narcotized rats showed that intravenous infusion of GABA derivative lithium hydroxybutyrate induced different changes in hemodynamic and respiratory parameters in animals with high and low resistance to hypoxia. Rats highly resistant to hypoxia better tolerated lithium hydroxybutyrate treatment compared to low resistant animals.

Key Words: *lithium hydroxybutyrate; hemodynamics; respiration; individual resistance to hypoxia; ultrasound*

There is a correlation between individual resistance to hypoxia and resistance to other factors. Rats highly resistant (HR) to hypoxia are more resistant to narcotic drug nembutal compared to low resistant (LR) animals [2]. Published data show that resistance to hypoxia correlates with seizure resistance [1,2]. GABA plays an important role in the formation of respiratory rhythm, but its effects vary due to the existence of several receptor types involved in the regulation of respiration in central structures [10,11]. $GABA_B$ receptors in the solitary tract play a role in the regulation of cardiovascular function [13,14]. It can be hypothesized that the reaction of animals to exogenous GABA-positive compounds (*e.g.*, lithium hydroxybutyrate) correlates with individual resistance to hypoxia. Lithium hydroxybutyrate is a GABA-positive hydroxyderivative and GABA metabolite. This compound more easily crosses the blood-brain barrier compared to GABA [7].

Here we studied the effect of GABA derivative lithium hydroxybutyrate on hemodynamics and respiration in rats with different resistance to acute hypoxia.

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MATERIALS AND METHODS

Acute experiments were performed on 53 male outbred albino rats weighing 350-400 g. The animals were intraperitoneally narcotized with nembutal in a dose of 40 mg/kg. Blood pressure was recorded in the femoral artery using a micromanometer. Blood flow rate in the ascending aorta was measured with a miniature ultrasound transducer fixed at the end of a catheter (diameter 0.6. mm). The operating frequency was 27 mHz [5]. A transducer was introduced into the aorta through the right carotid artery. The data on blood flow rate in the ascending aorta and blood pressure were processed using an analog-digital device to estimate total peripheral vascular resistance (mm Hg/cm/sec). Cardiac output and stroke volume were also measured using an electronic device. Heart rate was recorded with a cardiotachometer triggered by the aortic pulse wave. Respiratory movements of the thorax w0012ere recorded with a tensiometric sensor. Blood flow velocity and volumetric flow rate in the carotid and femoral arteries of 13 rats

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were measured with ultrasound transducers (inner diameters 0.7 and 0.5 mm). Carotid artery resistance was estimated in some animals. Electromyogram (EMG) was recorded in 16 rats using bipolar nichrome electrodes (diameter 0.3 mm) fixed at the abdominal surface of the diaphragm cupola. The amplitude of diaphragmatic discharge and length of respiratory cycle phases were measured.

The individual resistance of animals to hypoxia was estimated during inspiration of a gas mixture containing 3% O_2 and nitrogen by the latency of apnea [8,9]. Gas mixture supply was then stopped and if breathing did not restore spontaneously within 1-1.5 min artificial lung ventilation was started. Lung ventilation was stopped with the appearance of breathing. The animals with apnea latency of 1-4, more than 9, and 5-8 min after the start of hypoxic exposure were classified as LR, HR, and intermediate resistant (IR), respectively [2,8]. Twenty-two rats repeatedly breathed the gas mixture 30-40 min after treatment with lithium hydroxybutyrate to estimate its effect on the resistance to acute hypoxia.

GABA derivative lithium hydroxybutyrate was injected intravenously in a dose of 750 mg/kg (38% solution) to modulate activity of the GABAergic system.

The significance of differences was estimated by Student's t test ($p<0.05$).

Breathing 3% O_2 is life incompatible. The survival time (*i.e.*, resistance to severe hypoxia) differed in experimental animals. In our experiments apnea was observed 1-30 min after breathing of a gas mixture.

RESULTS

Of 53 animals, 28 (53%), 18 (34%), and 7 (13%) were HR, LR, and IR to hypoxia, respectively.

Systemic blood pressure in HR and LR rats decreased to 50 and 30 mm Hg, respectively, after intravenous infusion of lithium hydroxybutyrate ($p<0.05$). Our results are consistent with published data that intravenous infusion of GABA reduces blood pressure [7]. Total peripheral vascular resistance decreased to 20-30% of the baseline level. We revealed a transient decrease in blood pressure and total peripheral vascular resistance. It was observed over 1.5-2 min of treatment. The test parameters in HR and LR animals differed in the follow-up period. Blood pressure and total peripheral vascular resistance in most HR rats increased by the end of treatment with lithium hydroxybutyrate and remained above the normal for more than 20-30 min (Fig. 1, *a*). Blood pressure in some HR animals slightly decreased after 30-40 min. Blood pressure and total peripheral vascular resistance in LR rats returned to normal by the end of lithium hydroxybutyrate administration, but then progressively decreased and after 30-40 min corresponded to 50% of the baseline level (Fig. 2, *a*).

After intravenous infusion of lithium hydroxybutyrate cardiac output in HR rats increased by 60

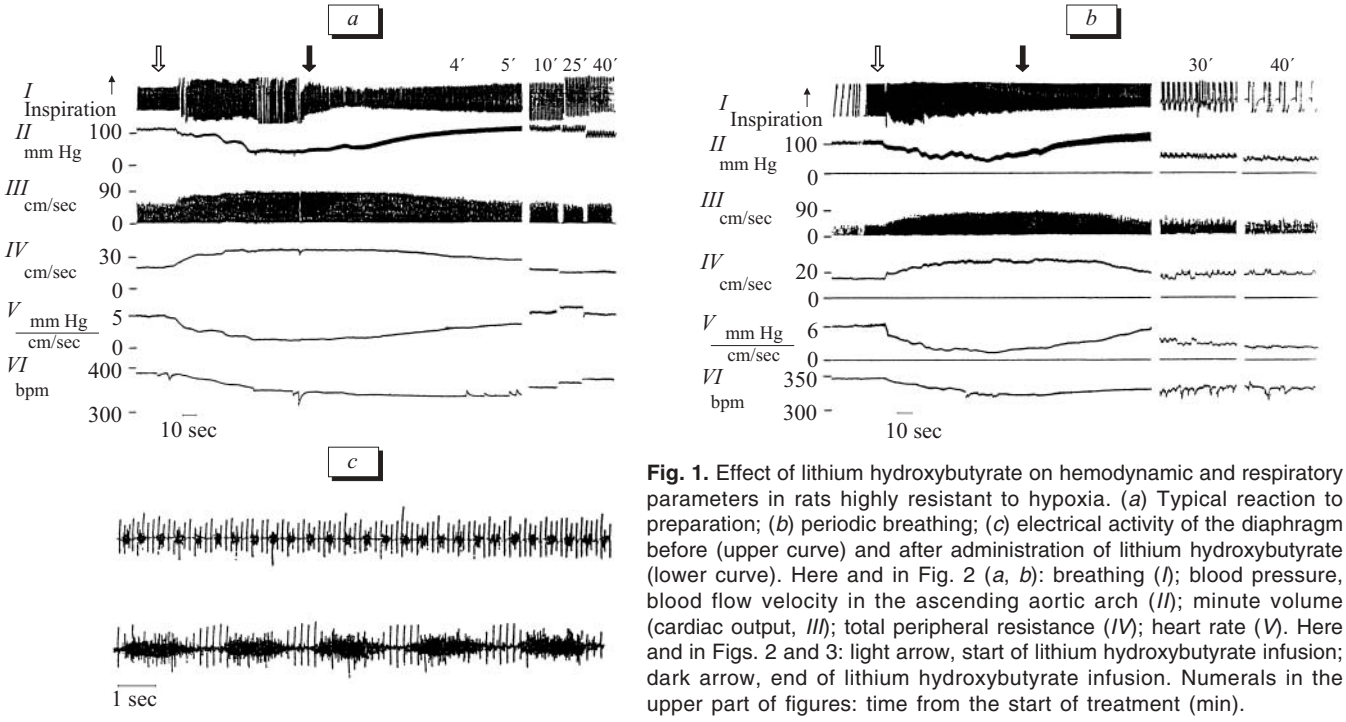

Fig. 1. Effect of lithium hydroxybutyrate on hemodynamic and respiratory parameters in rats highly resistant to hypoxia. (*a*) Typical reaction to preparation; (*b*) periodic breathing; (*c*) electrical activity of the diaphragm before (upper curve) and after administration of lithium hydroxybutyrate (lower curve). Here and in Fig. 2 (*a*, *b*): breathing (*I*); blood pressure, blood flow velocity in the ascending aortic arch (*II*); minute volume (cardiac output, *III*); total peripheral resistance (*IV*); heart rate (*V*). Here and in Figs. 2 and 3: light arrow, start of lithium hydroxybutyrate infusion; dark arrow, end of lithium hydroxybutyrate infusion. Numerals in the upper part of figures: time from the start of treatment (min).

(50-70%). In some animals cardiac output increased by 100% (Fig. 1, *a*). Cardiac output in LR rats increased by 40% ($p<0.05$, Fig. 2, a). The decrease in cardiac output in LR animals was accompanied by respiratory arrest (Fig. 2, *b*).

The increase in cardiac output is probably associated with not only specific effect of lithium hydroxybutyrate, but also scheme of treatment with the test preparation (hypertonic solution) [4]. The increase in cardiac output can be related to fluid transition from tissue to blood along the osmotic gradient [3], which increased plasma volume. The extra blood volume is directed to various vessels including carotid and femoral arteries. The increase in cardiac output probably contributed to rapid recovery of blood pressure. Cardiac output in HR animals progressively decreased starting from the 3rd minute after lithium hydroxybutyrate administration. Cardiac output returned to normal after 10 min and remained unchanged in the follow-up period (30-40 min, Fig. 1, *a*). Cardiac output in LR rats increased by 40% during administration of lithium hydroxybutyrate, but progressively decreased starting from the 3rd minute. Cardiac output in these animals was 30% below the baseline level after 10 min ($p<0.05$) and continued to decrease in the follow-up period (Fig. 2, *a*).

Heart rate in HR and LR rats decreased by 40-50 and 100 bpm, respectively, after treatment with lithium hydroxybutyrate. Heart rate remained low for 10 min, but returned to normal in the follow-up period. Heart rate in HR rats did not differ from normal after 40 min. Heart rate in LR animals did not completely return to normal. Cardiac output increased, while heart rate decreased in most HR and LR rats. These changes reflect a transient increase in stroke volume. It can be concluded that in HR rats contractile function of the myocardium was sufficiently high at all terms after treatment and in LR animals was adequate for at least 10 min, which corresponded to high level of cardiac output.

In some experiments lithium hydroxybutyrate was injected twice in a half dose, because of significant decrease in blood pressure and respiratory dysfunction. Repeated administration of the test preparation also changed blood pressure, total peripheral vascular resistance, cardiac output, and heart rate. However, these changes persisted for a shorter period of time. The exception was heart rate, which continued to decrease after repeated treatment with lithium hydroxybutyrate.

Published data show that GABA produces a dose-dependent inhibitory effect on respiration [7, 10,11]. In our experiments a GABA derivative lithium hydroxybutyrate produced various respiratory changes in animals with different resistance to hypoxia. The amplitude of respiratory movements increased over the first 1.5-2 min after administration

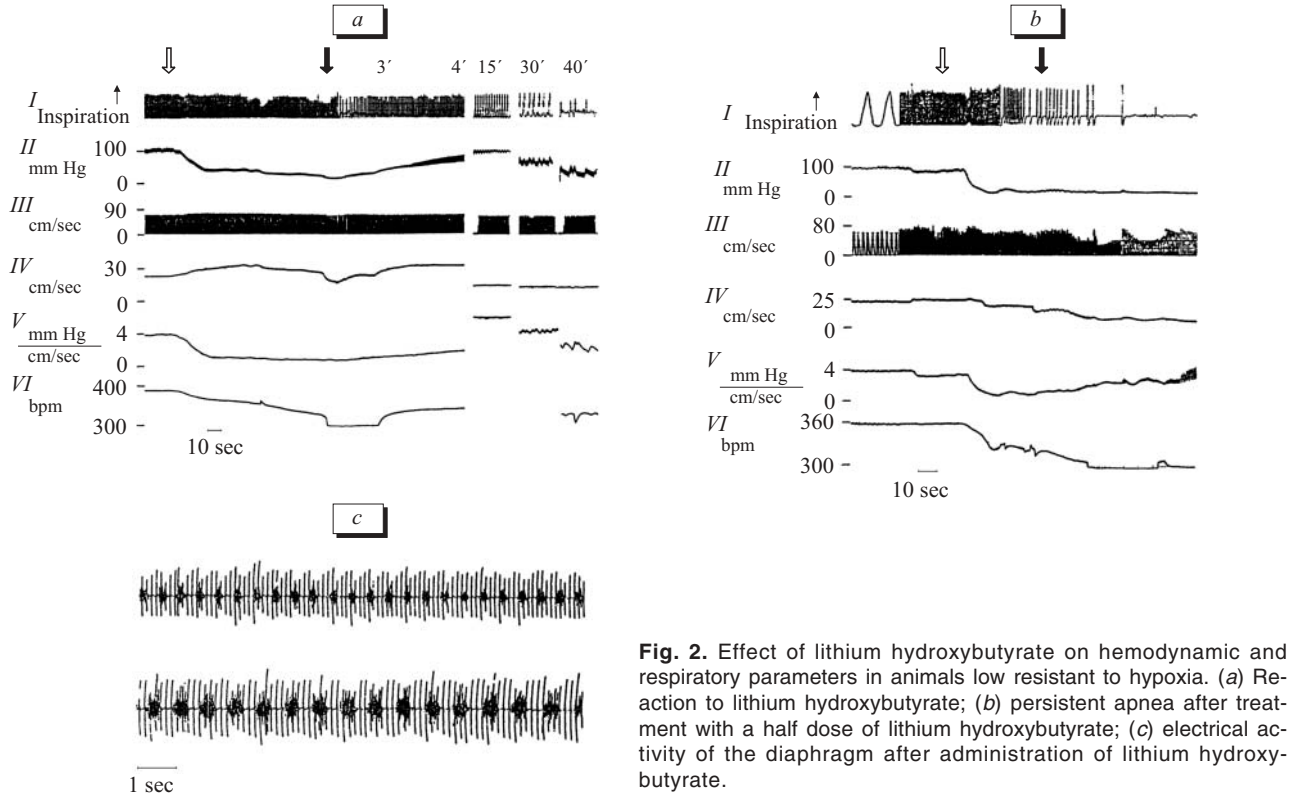

Fig. 2. Effect of lithium hydroxybutyrate on hemodynamic and respiratory parameters in animals low resistant to hypoxia. (*a*) Reaction to lithium hydroxybutyrate; (*b*) persistent apnea after treatment with a half dose of lithium hydroxybutyrate; (*c*) electrical activity of the diaphragm after administration of lithium hydroxybutyrate.

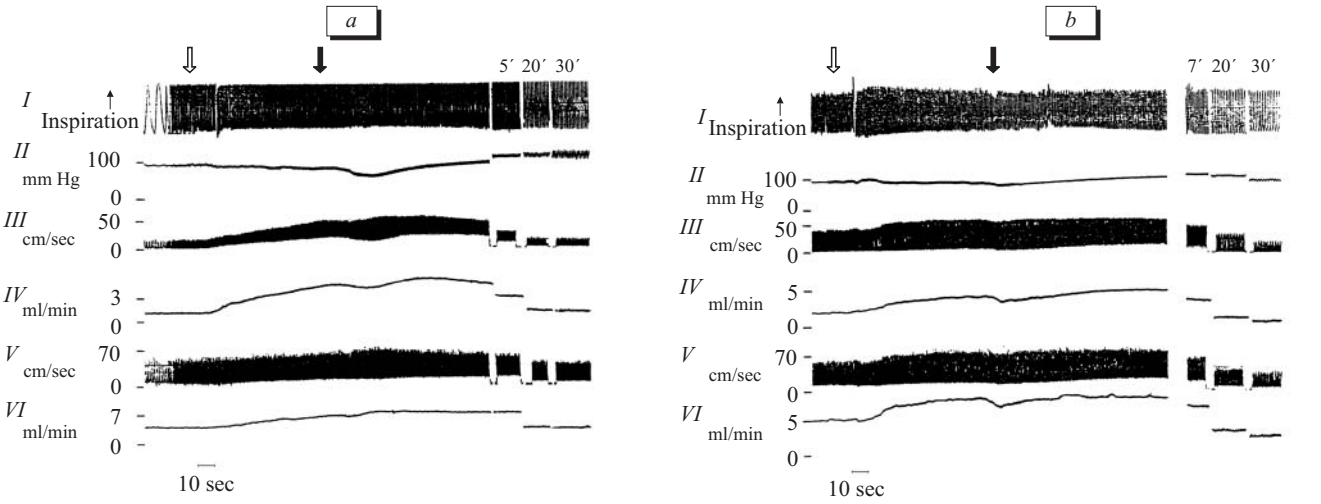

Fig. 3. Blood flow in the carotid and femoral arteries of rats highly (*a*) and low resistant to hypoxia (*b*) receiving lithium hydroxybutyrate. Respiration (*I*), blood pressure (*II*); blood flow velocity in the femoral artery (*III*); volumetric flow rate in the femoral artery (*IV*); blood flow velocity in the carotid artery (*V*); volumetric flow rate in the carotid artery (*VI*).

of the test preparation (Fig. 1, *a*). Bradypnea in most HR rats was observed 2-3 min after administration of lithium hydroxybutyrate. Slowness of breathing was most pronounced 20-30 min after treatment with the test preparation (2-2.5-fold decrease in respiratory rate). However, the amplitude of respiratory movements remained above the normal in this period. This type of breathing was previously revealed in HR rats with severe acute hypoxia produced by long-term breathing a hypoxic mixture [8]. The respiratory rate in 3 particularly resistant rats decreased, but returned to normal 20-30 min after repeated administration of lithium hydroxybutyrate. Periodic apnea (2 inspirations and expiratory pause) was observed in 6 HR rats (21%, Fig. 1, *b*). Periodic breathing was revealed in animals with low blood pressure. Previous experiments demonstrated higher incidence of periodic breathing in response to administration of lithium hydroxybutyrate [10]. Treatment with lithium hydroxybutyrate was followed by persistent apnea in 3 HR rats (1.7%).

In LR rats bradypnea was observed 1-1.5 min after the start of treatment with lithium hydroxybutyrate. These changes were usually accompanied by a decrease in the amplitude of respiratory movements (Fig. 2, *a*). Persistent apnea developed in 7 LR rats (38%). Respiration did not return to normal even under conditions of artificial lung ventilation. Some animals exhibited apnea after treatment with a half dose of lithium hydroxybutyrate (Fig. 2, *b*).

In IR rats bradypnea was also observed. Transient apnea (50-60 sec) was typical of 5 IR animals. Respiration recovered in the follow-up period.

Our results are consistent with published data on the effect of posthemorrhagic hypoxia [4]. Under conditions of compensated blood loss, intravenous infusion of lithium hydroxybutyrate (20% solution) after a short-term decrease in blood pressure and reduction of portal blood flow was followed by a progressive increase in hemodynamic parameters. Intravenous infusion of hypertonic lithium hydroxybutyrate to decompensated posthemorrhagic animals (*i.e.*, LR rats) produced an irreversible decrease in systemic blood pressure and other hemodynamic parameters, which resulted in death.

Recording of diaphragmatic EMG showed that inspiration of a hypoxic mixture is accompanied by an increase in the amplitude of discharge. Treatment with lithium hydroxybutyrate was followed by an increase in the amplitude of EMG in all HR rats and 50% LR animals. The amplitude of diaphragmatic discharge decreased in the remaining LR rats (50%), which was accompanied by a change in respiratory rate and persistent apnea. We revealed lengthening of the inspiratory phase. It should be emphasized that repeated injection of lithium hydroxybutyrate was effective in this respect. The inspiratory phase in HR and LR rats increased by 400 (Fig. 1, *c*) and 200%, respectively (Fig. 2, *c*). The duration of the expiratory phase remained practically unchanged after systemic administration of lithium hydroxybutyrate.

EMG recording showed that lithium hydroxybutyrate produces opposite changes in the amplitude of diaphragmatic discharge in rats with different resistance to hypoxia. Moreover, these animals differed in post-treatment prolongation of the inspiratory phase.

Published data show that lithium hydroxybutyrate increases the resistance of rats to moderate

hypoxia (10% O_2) [12]. Twenty-two rats were tested for apnea produced by breathing a hypoxic mixture with 3% O_2 before and after administration of lithium hydroxybutyrate. Apnea developed more rapidly in HR and LR animals receiving the test preparation. In LR rats apnea was observed several seconds after the start of treatment. These data indicate that administration of lithium hydroxybutyrate is followed by a significant decrease in the resistance of animals to severe hypoxia. Our previous studies showed that after repeated breathing a hypoxic gas mixture (3% O_2) not accompanied by administration of lithium hydroperoxide, most HR rats retain high resistance to hypoxia. Some HR animals became IR, but nor LR [8].

GABA plays an important role in the regulation of cerebral blood flow. Published data show that GABA decreases the resistance of cerebral vessels, but increases the volumetric flow rate [6].

Study of blood flow in the carotid and femoral arteries revealed 7 HR and 6 LR animals. Blood flow in the carotid artery of HR and LR rats increased by 1.5-2 times after intravenous infusion of lithium hydroperoxide. These changes were accompanied by an increase in cardiac output. The resistance in carotid arteries decreased under these conditions. The reduction of cardiac output 7-10 min after treatment was accompanied by a decrease or increase in blood flow, which depended on the resistance to hypoxia. Blood flow in HR rats returned to normal 20-30 min after treatment and remained unchanged until the end of the experiment (Fig. 3, *a*). Carotid blood flow in LR animals decreased to 50-60% of the baseline level 20-30 min after treatment (Fig. 3, *b*).

Femoral blood flow increased by 2.5-3 times immediately after administration of lithium hydroxybutyrate (10-fold increase in 1 rat), but decreased practically to zero 5-7 min after treatment.

Our results indicate that changes in hemodynamic and respiratory parameters produced by a GABA derivative lithium hydroxybutyrate depend on the resistance of animals to hypoxia. Test preparation is more tolerable for HR animals.

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