# Formation of Periodic Apneustic Breathing by Activation of the GABA-ergic System of the Brain

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Gammaaminobutyric acid (GABA), the most important inhibitory transmitter in the central nervous system, also plays an important role in the functioning of the respiratory center of the brain stem. GABA-ergic terminals have been found in many nuclei which are traditionally considered to be the morphological substrate of the central respiratory apparatus [6, 12]. GABA has an inhibitory effect on expiratory and inspiratory neurons of the pons (pneumotaxic center) and medulla oblongata (nucleus ambiguus and nucleus of the solitary tract) [3,4,11]. The central GABA-ergic mechanisms are thought to be involved in the development of certain forms of respiratory pathology in newborns [7]. Since pronounced hypoxia, which usually leads to a secondary respiratory depression, is probably connected with an increase of the GABA content in the brain stem [8], the release of this neurotransmitter in response to certain influences, for instance, in hypoxia, may induce the development of pathological breathing in several diseases [9,13]. In our previous paper, focusing mainly on methodology [2], we described the overall picture of changes in breathing and in several hemodynamic parameters in narcotized cats in hydroxybutyrate- or lithium-induced periodic apneustic breathing.

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The aim of the present study was to investigate the mechanisms underlying the disturbances in breathing rhythm for activation of the brain GABA-ergic system and the involvement of the afferent system of the lungs in these processes.

## MATERIALS AND METHODS

The experiments were carried out on male and female outbred cats weighing 2.25-4.25 kg under nembutal narcosis (40 mg/kg, i.p.). The rectal temperature was measured immediately after narcosis and then was maintained with an electric heater within 37.5-38.5°C with an accuracy of ±0.5°C. Tracheotomy was performed in the upper third of the trachea. A cannula connected to transducers was inserted in the trachea for registration of the respiratory parameters. For recording of the intraesophageal pressure a catheter with a water-filled elastic balloon connected to a low-pressure transducer was introduced into the esophagus. The systemic arterial pressure and heart rate were registered with a catheter inserted in the femoral artery. Blood for determination of the parameters of the acid-base balance (ABB) and blood gases tension was also drawn through this catheter. The composition of the arterial blood was analyzed with an ABL-330 device (Radiometer International). For a study of the dynamics of pH, $pO_2$, and $pCO_2$ the arterial blood was pumped into a DS 67101 flow cuvette with correspondent electrodes. The cuvette was thermostated at 37°C with

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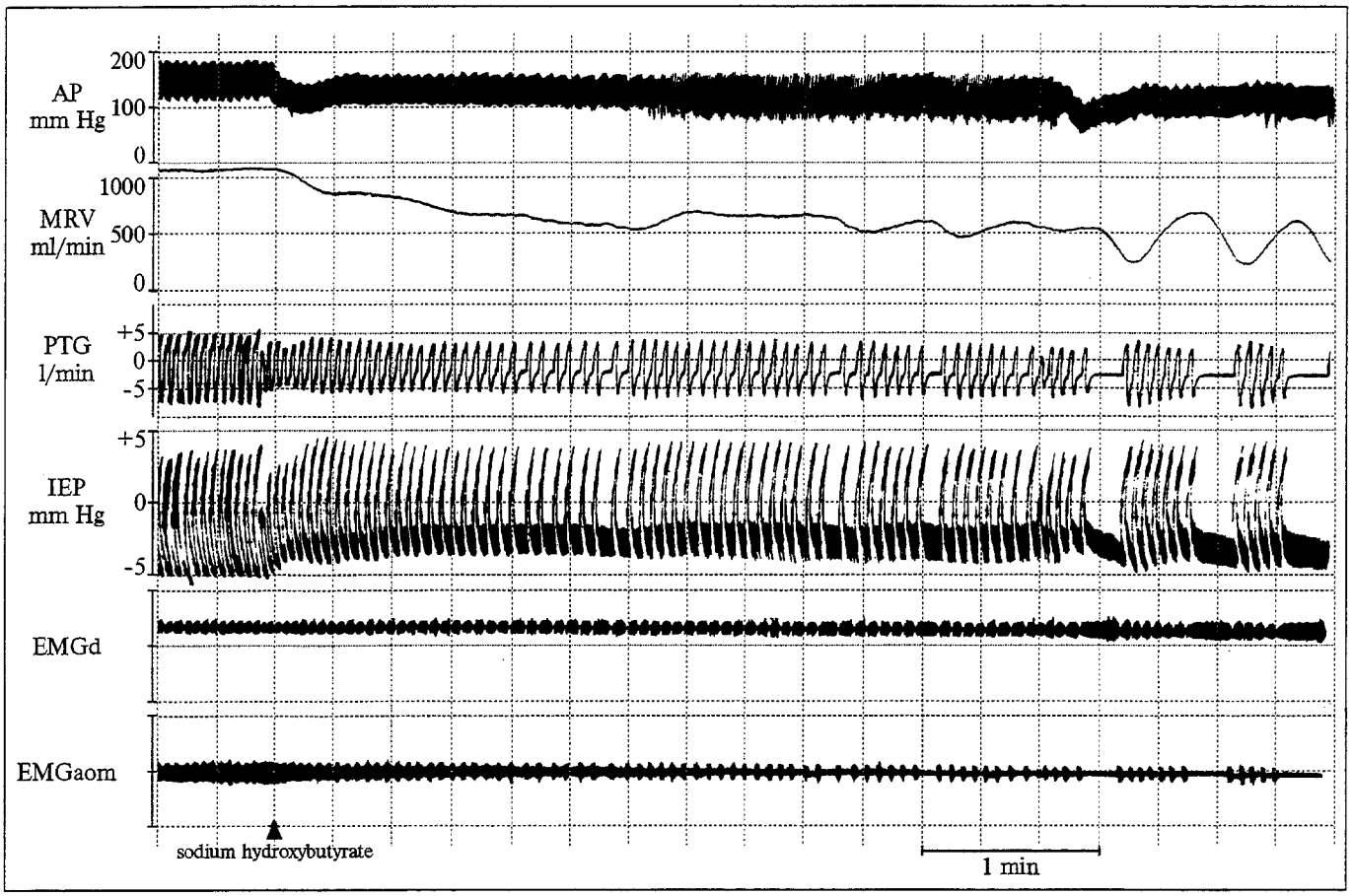


Fig. 1. Dynamics of respiratory and circulatory parameters after administration of sodium hydroxybutyrate. From top to bottom: AP: arterial pressure in the systemic circuit, MRV: minute respiratory volume, PTG: pneumotachogram, IEP: intraesophageal pressure, EMGd: electromyogram of the diaphragm, EMGaom: electromyogram of the abdominal oblique muscles.

a VTS13c thermostat (all equipment from Radiometer). After amplification and treatment, the parameters of pH, $pO_2$, and $pCO_2$ were recorded with KSP-4 direct writers. Electromyograms (EMG) of the costal diaphragm, intercostal (8-10 intercostal space) and interchondral (6-8 intercostal space) muscles, and abdominal muscles (rectum and oblique muscles) were recorded with an M-42 electromyograph (Hungary) using hooked electrodes. Bilateral vagotomy was performed at the level of the neck. The preparations were injected through the catheter in the femoral vein. The breathing parameters (breathing rate, BR, respiratory volume, RV, minute respiratory volume, MRV), the hemodynamic parameters (arterial pressure, AP, and heart rate, HR) and intraesophageal pressure (IEP) were recorded with a domestic MKh-01 polygraph. The EMG of the respiratory muscles and the breathing and hemodynamic parameters were recorded with a 14-channel Tesla EAM 500 magnetograph (Czechoslovakia) for further treatment and registration. Sodium hydroxybutyrate was used for the experiments (200 mg/kg, i.v.). Hydroxybutyrate is a hydroxyderivative and metabolite of GABA, which more easily crosses the blood-brain barrier and may interact with the GABA-ergic receptors in the brain. The data were processed statistically using Student's $t$ test. A difference with $p<0.05$ was considered as reliable.

## RESULTS

Previously we demonstrated [2] that systemic hydroxybutyrate infusion rapidly (within 1-3 min) caused bradypnea (Table 1), usually followed by an irregular breathing rhythm in more than 80% of cases. The breathing formed under these conditions was periodic apneustic breathing with long inspiratory efforts [2]. It should be noted that rapid injection of hydroxybutyrate may result in respiratory and cardiac depression immediately post-injection, probably through a chemoreflex mechanism, because of the alkaline pH (about 7.85) of the 20% hydroxybutyrate solution used (Fig. 1).

Apart from bradypnea, changes in the depth of breathing also occurred. The direction of changes in respiratory volume evidently depended on the degree

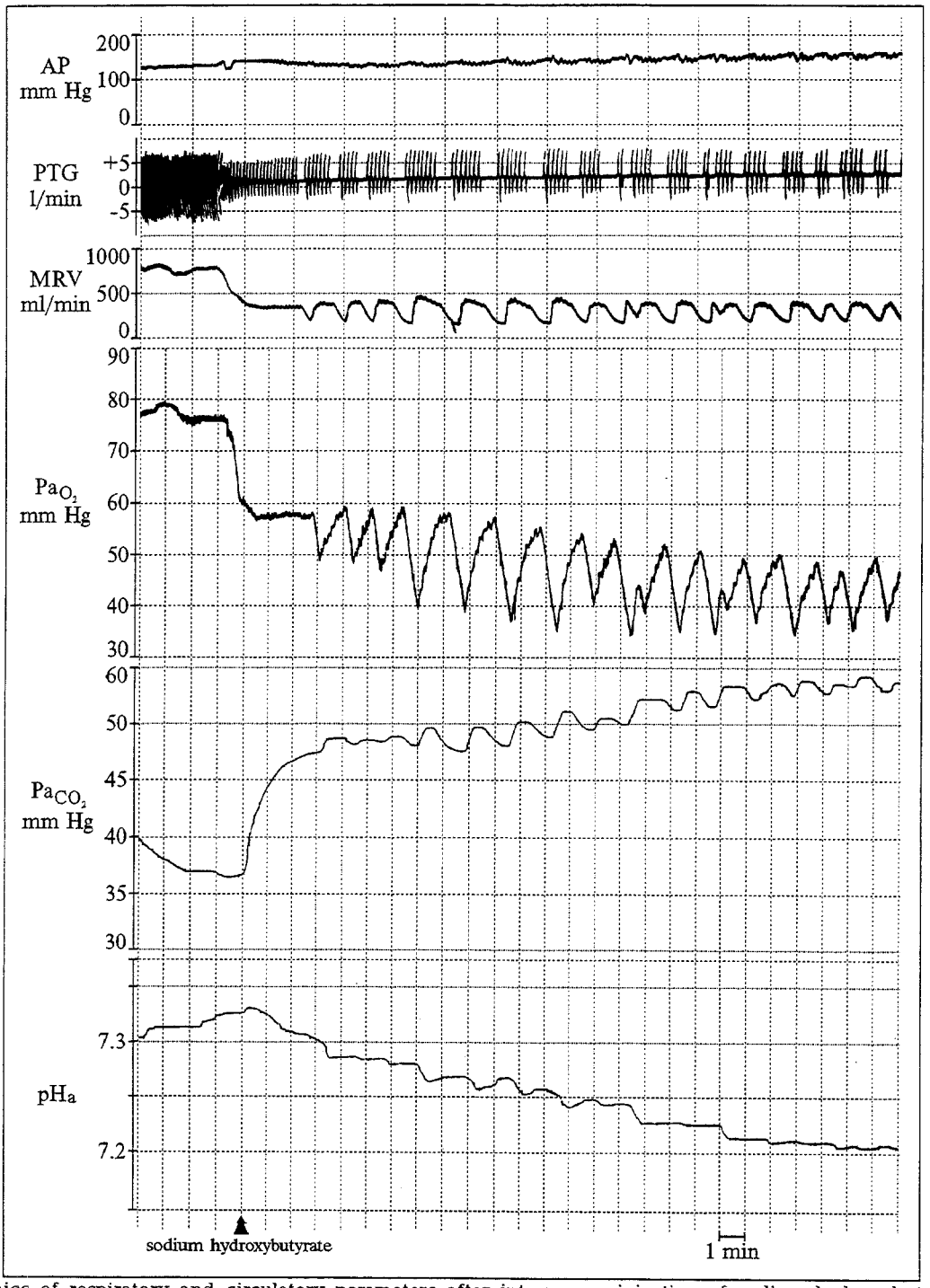


Fig. 2. Dynamics of respiratory and circulatory parameters after intravenous injection of sodium hydroxybutyrate. From top to bottom: AP: arterial pressure in the systemic circuit, PTG: pneumotachogram; MRV: minute respiratory volume, $P_aO_2$ oxygen tension in arterial blood; $P_aCO_2$: $CO_2$ tension in arterial blood, $pH_a$: reaction of arterial blood.

of respiratory rhythm depression: the depth of respiration increased in the case of more pronounced and decreased in less pronounced bradypnea. The mean value of this parameter exhibited a marked tendency to increase (Table 1). Judging from the pneumotachogram, the decrease of the respiration depth may be related to a reduced peak rate of the inspiratory flow (Fig. 1). The above-mentioned changes in the depth of breathing led to a more than 2-fold decrease in the ventilation of the lungs (Table 1). In abrupt periodic breathing characterized by the appearance of 3-5 respiratory cycles in a "packet", a certain modulation of the amplitude between the cycles was observed, the amplitude being maximal in the 2nd-3rd cycles with its subsequent decrease. In such bradypnea with a mean respiratory

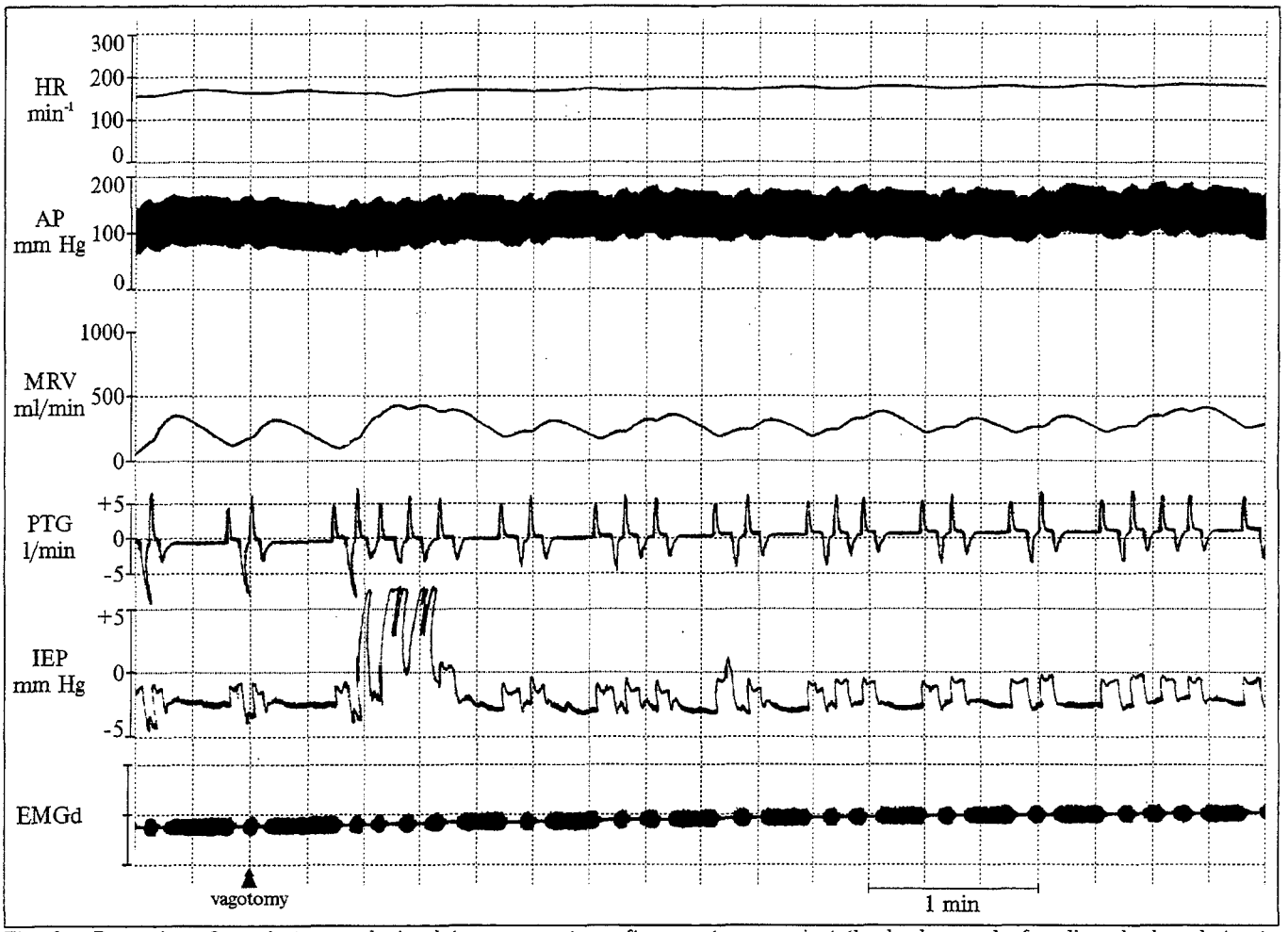


Fig. 3. Dynamics of respiratory and circulatory parameters after vagotomy against the background of sodium hydroxybutyrate (30 min). From top to bottom: HR: heart rate; AP: arterial pressure in the systemic circuit; MRV: minute respiratory volume; PTG: pneumotachogram; IEP: intraesophageal pressure; EMGd: electromyogram of the diaphragm.

rate of 3-5 cycles per minute the reduction of respiratory depth was not pronounced in comparison with more frequent breathing of the same periodic, inspiratory pattern. It seems likely that in bradypnea the influence of chemoreceptor impulsation on the respiratory center is enhanced, thus compensating for primary reduction of the depth of respiration.

The holding of the breath in the above-described disturbance of the rhythm of breathing is inspiratory in nature: during these periods a continuous impulse activity of the expiratory muscles (external intercostals and diaphragm) was registered and the intraesophageal pressure was lowered and close to the minimal value observed in original breathing. In some cases additional inspiratory activation was observed at the end of long inspiratory phases, which manifested itself as an additional rarefaction corresponding to enhanced activity of the inspiratory muscles (Fig. 1).

It is characteristic that the activity of the expiratory muscles (internal intercostals and abdominal muscles) was not depressed after the pathological breathing set in, as it might be assumed in view of the reciprocal relationships between the expiratory and inspiratory parts of the breathing apparatus. On the contrary, in some cases, while initially absent, such activity even arises. It may be noted that the amplitude of impulses in the expiratory muscles is usually maximal in the first burst after prolonged inspiratory breath-holding.

We contend that the changes in the respiratory pattern cannot be attributed to alterations in the systemic hemodynamics, since the tendency toward a reducted HR and lowered AP (the only alterations observed) was not enough to cause marked disturbances in the rhythm and pattern of breathing (Table 1).

Periodic changes in MRV affect the arterial blood components as follows: the changes were most pronounced in $pO_2$, less pronounced in $pCO_2$ and lowest of all in pH. Similarly to MRV, the mean values of $pO_2$ and $pCO_2$ in periodic breathing were, respectively, twice or half as much as the initial values (Fig. 2). The pH under these conditions is shifted by 0.1-0.2 toward acidosis in comparison with the control value. A certain delay in the variation of the

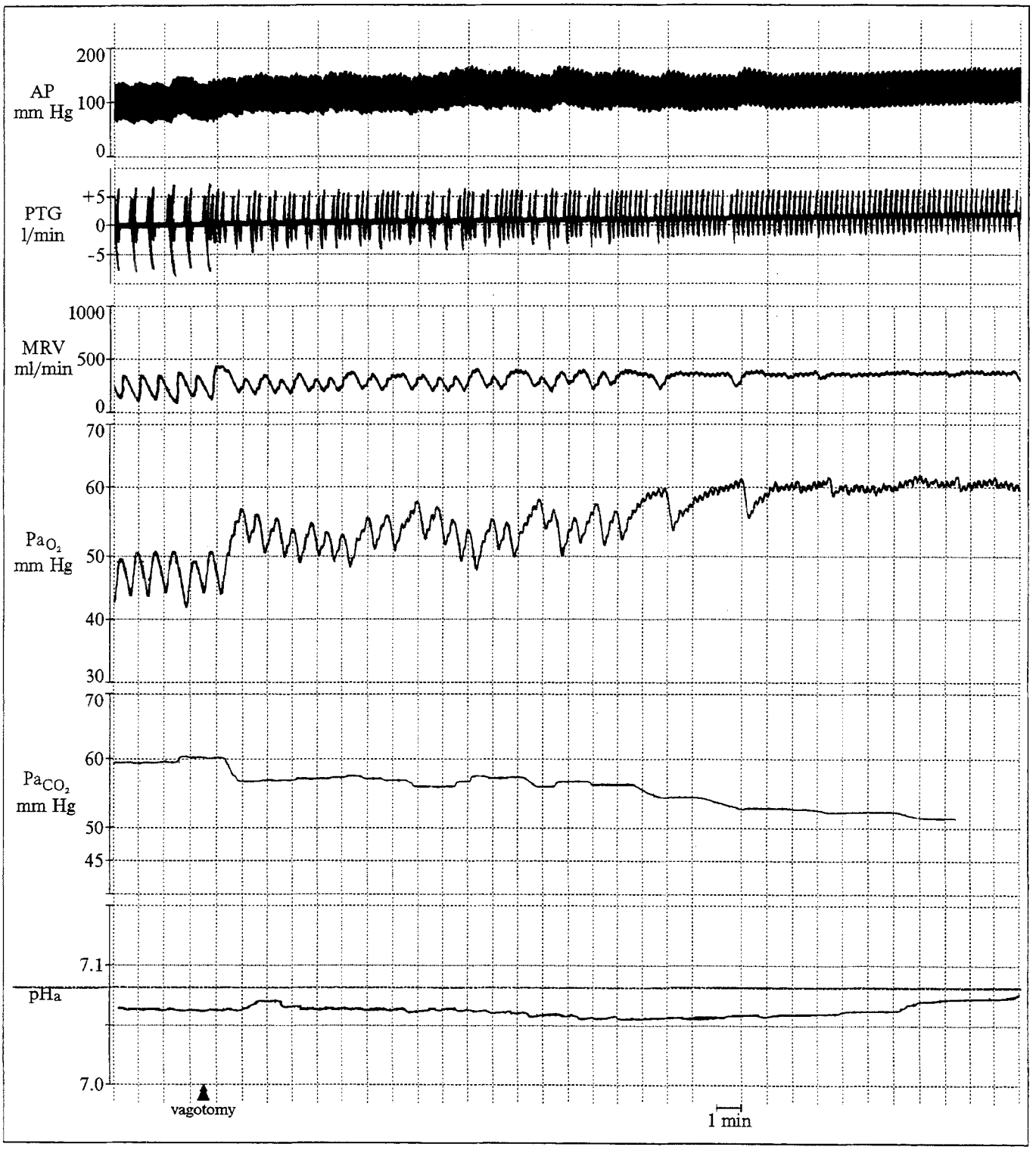


**Fig. 4.** Dynamics of respiratory and circulatory parameters against the background of sodium hydroxybutyrate. From top to bottom: HR: heart rate; AP: arterial pressure in the systemic circuit; PTG: pneumotachogram; MRV: minute respiratory volume; $P_aO_2$ oxygen tension in the arterial blood; $P_aCO_2$: $CO_2$ tension in the arterial blood; $pH_a$: reaction of the arterial blood.

blood gases content from the changes in MRV supports our assumption that in the "packet" of respiratory movements the cycle which corresponds to the maximal decrease of $pO_2$ in the arterial blood has the maximal amplitude.

Since vagotomy is a commonly used experimental procedure for reducing BR and affecting the respiratory rhythm due to interruption of the Hering-Breuer reflex arc, we performed bilateral vagotomy after pathological breathing had set in, thus antici-

**TABLE 1.** Respiratory and Circulatory Parameters in Narcotized Cats after Intravenous Hydroxybutyrate Administration (200 mg/kg)

| Parameter | Time, min | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | initial value | 1 | 3 | 5 | 10 | 15 | 20 | 25 | 30 | 45 | 60 | 75 |
| HR, beats/min | 218±21 | 206±23 | 193±23 | 186±25 | 185±27 | 184±27 | 170±17 | 169±14 | 167±12 | 209±22 | 215±26 | 233±28 |
| AP, mm Hg | 121±11 | 118±17 | 113±13 | 105±16 | 108±15 | 110±15 | 106±14 | 102±12 | 103±14 | 123±18 | 135±16 | 125±13 |
| MRV, ml/min | 599±81 | 453±100 | 395±68 | 306±73* | 282±86* | 244±86* | 224±43* | 205±51* | 194±45* | 155±23* | 244±50* | 189±22* |
| BR, cycles/min | 27.0±3.7 | 17.2±2.4 | 11.5±2.0* | 8.2±1.1* | 6.6±1.2* | 5.2±1.1* | 5.8±1.0* | 4.8±0.8* | 5.1±0.7* | 4.6±0.7* | 4.9±1.3* | 4.5±1.5* |
| RV, ml | 26.3±3.8 | 32.4±8.2 | 34.9±5.9 | 33.6±5.6 | 37.9±8.4 | 48.7±20 | 52.6±21 | 39.6±7.7 | 37.8±6.5 | 32.8±6.4 | 45.7±9.0 | 43.5±2.5 |

Note. The differences are reliable ($p<0.05$).

pating an aggravation of the disturbances in the breathing rhythm and further inspiratory depression. However, vagotomy either produced no marked alterations in the respiratory and circulation parameters (Fig. 3) or even promoted the restoration of more regular breathing (Fig. 4). The latter resulted in a general MRV increase and consequently in a certain improvement of the respiratory parameters of the arterial blood. It is also highly characteristic for several thrusts of IEP to appear, reflecting the motor activity of the esophagus and stomach.

It is still not clear why vagotomy performed against the background of hydroxybutyrate administration lost its ability to change the breathing rhythm. It may be assumed that systemic administration of hydroxybutyrate affects the GABA-ergic structures of the medulla oblongata, including the nucleus of the solitary tract, where the afferent vagal terminals are located [10]. Such an intervention may interfere with the afferent vagal transmission to the neurons of the respiratory center, thus producing "central vagotomy". This assumption is in conformity with the data concerning the significant content of GABA in the nucleus of the solitary tract in the cat brain [5]. This central vagotomy may be mediated through activation of GABAb receptors, which are involved in presynaptic inhibition [1].

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