Involvement of the GABA-Ergic and N-Cholinergic Systems in the Establishment of Terminal Respiration

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In experiments with Nembutal-anesthetized cats, in which lobeline, picrotoxin, tubocurarine, and naloxone were injected into the fourth brain ventricle, respiratory disturbances resulting from activation of the central N-cholinergic receptors by lobeline injected when central $GABA_A$ receptors were blocked by picrotoxin led to respiration of the gasping type. After naloxone was administered additionally, the normal rhythmic pattern of breathing was restored.

Key Words: *regulation of respiration; lobeline; picrotoxin; tubocurarine; naloxone; GABA-ergic and cholinergic systems*

Blockade of the central N-cholinergic receptors with tubocurarine has been shown to abolish the inhibition of respiratory activity caused by sodium oxybutyrate (or by ethanol), indicating that the cholinergic system is actively involved in the regulation of respiration [2]. It has also been found that N-cholinergic receptors exist at the respiratory center and that GABA-ergic and cholinergic neurons are sequentially linked in certain brain regions [4,9,11-13].

The present study was designed to explore the interrelationship between the central N-cholinergic and GABA receptors during the formation of the respiratory rhythm and, in particular, the development of terminal respiration.

MATERIALS AND METHODS

The study was conducted on 68 cats (body weight 2.5-4.0 kg) under Nembutal anesthesia (40 mg/kg intraperitoneally) [2]. Lobeline at 800 nmol/kg or 3-4 μmol/kg, picrotoxin at 30 nmol/kg, tubocurarine at 130 nmol/kg, and naloxone at 20 nmol/kg were injected into the fourth ventricle of the brain in a volume of 50 μl (lobeline and naloxone in isotonic NaCl solution and picrotoxin and tubocurarine in 48% ethanol). In control tests, isotonic NaCl solution and 48% ethanol were similarly administered in the same volume as above (the effects from control injections are described in the preceding article [2]). Preliminarily, the carotid nerves were cut to denervate the carotid sinuses and thus prevent the respiratory effect of lobeline on the peripheral N-cholinergic receptors of carotid body cells. The cats were tracheotomized in order to record respiratory parameters and institute artificial ventilation.

RESULTS

In tests on 10 cats, designed to see how lobeline would affect respiration under normal conditions, injection of this drug at 800 nmol/kg was followed within 1 min by a sharp decrease in minute volume (MV) as a result of falls in both respiratory rate and respiratory volume, to the point of respiratory arrest for 10-15 min (the cats were on artificial ventilation during that period); 50 to 60 min after lobeline injection, spontaneous respiratory movements were restored and the respiratory rate and volume reached their baseline values (Fig.

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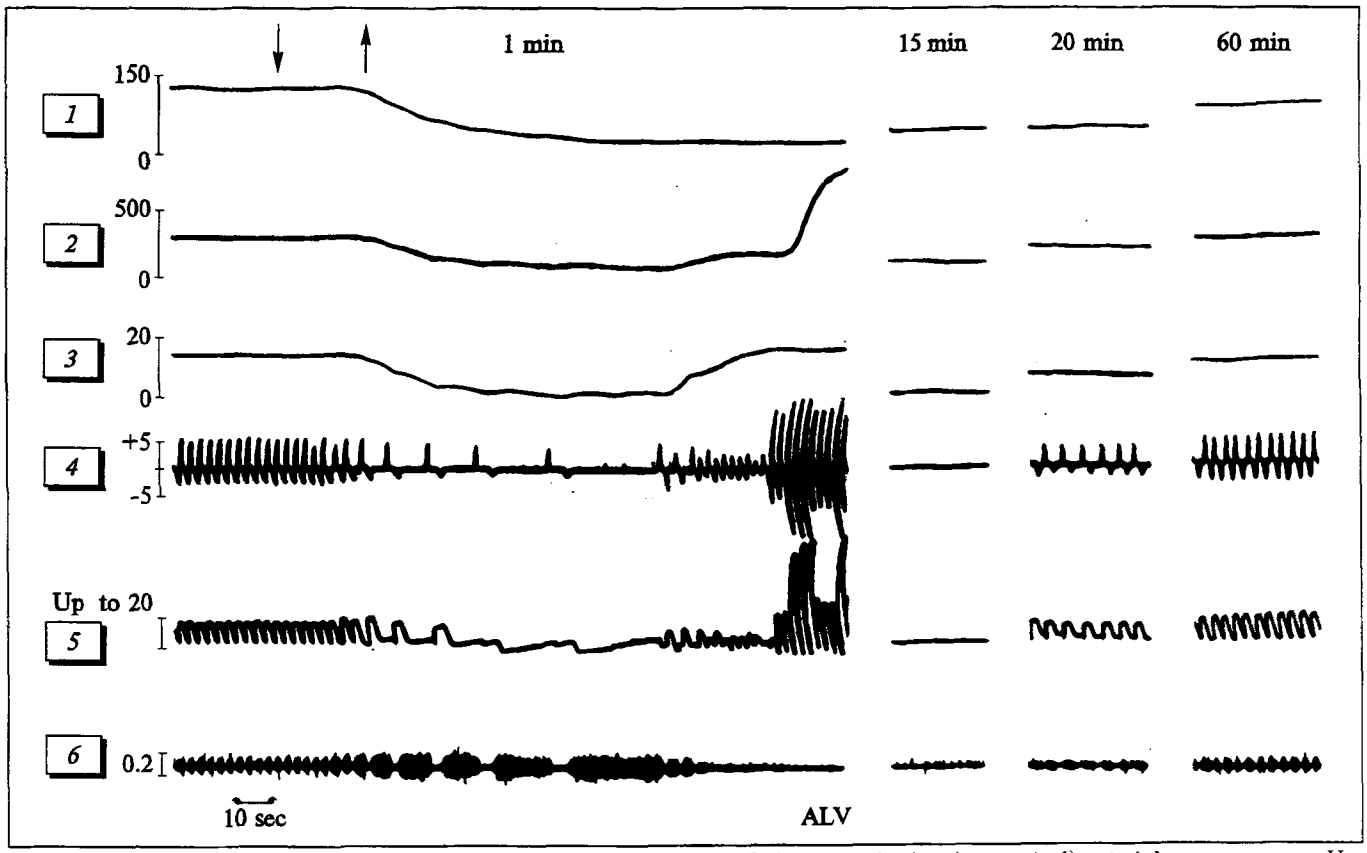

Fig. 1. Effect of intraventricularly administered lobeline (800 nmol/kg) on respiration in a cat. *1*) arterial pressure, mm Hg; *2*) minute volume, ml/min; *3*) respiratory rate, min^{-1}; *4*) pneumotachogram, liters/min; *5*) respiratory volume, ml; *6*) electromyogram of phrenic muscle, mV. *ALV*: artificial lung ventilation. The down and up arrows mark the beginning and end of lobeline injection, respectively; at top are times postinjection.

1). In tests on 7 other cats preinjected with tubocurarine before lobeline, the inhibitory effect of the latter on respiration was completely prevented.

In further tests with 10 cats in which the chloride channels functionally related to the $GABA_A$ receptors had been blocked with picrotoxin, one and even two lobeline injections failed to cause a significant fall in respiratory rate in any of the animals (Fig. 2); none of them exhibited any evidence of respiratory arrest.

All 12 cats given 3 or 4 lobeline injections at 5- to 10-minute intervals ceased to perform rhythmic respiratory movements and were observed to gasp for 15-60 min (during which time they were put on periodic artificial ventilation) (Fig. 3).

Thus, in this animal model of respiratory disturbances caused by activation of the central N-cholinergic receptors by lobeline after preliminary blockade of the central $GABA_A$ receptors with picrotoxin, gasping-like respiration occurred in all cases. In almost all of the cats that developed gasping after the second lobeline injection, spontaneous restoration of normal respiration was observed, whereas all the animals developing gasping after the third or fourth lobeline injection eventually died.

In subsequent tests with 8 cats, naloxone injected during gasping or after it, when respiratory movements had ceased, caused normal respiration to be restored at minute 4 or 5 postinjection, and all respiratory and hemodynamic parameters returned to their baseline values by minutes 25 to 30. As illustrated in Fig. 4, after three lobeline injections respiration stopped for 1 min against the background of a lowered minute volume, after which respiratory movements were resumed in a gasping-like form, while the injection of naloxone was followed by resumption of spontaneous rhythmic respiration in 5 min or so. It is also apparent in Fig. 4 that the activity of the internal intercostal muscles ceased and rapid short discharges occurred in the phrenic muscle, this being in line with the suggestion made by some workers that gasping is accompanied by inhibited activity of expiratory neurons and by synchronous rapid and short-term discharges of inspiratory neurons [15].

When the respiratory disturbances caused by lobeline through activation of the central N-cholinergic receptors before blockade of the chloride channels (central $GABA_A$ receptors) with picrotoxin were compared with those produced by it after the block-

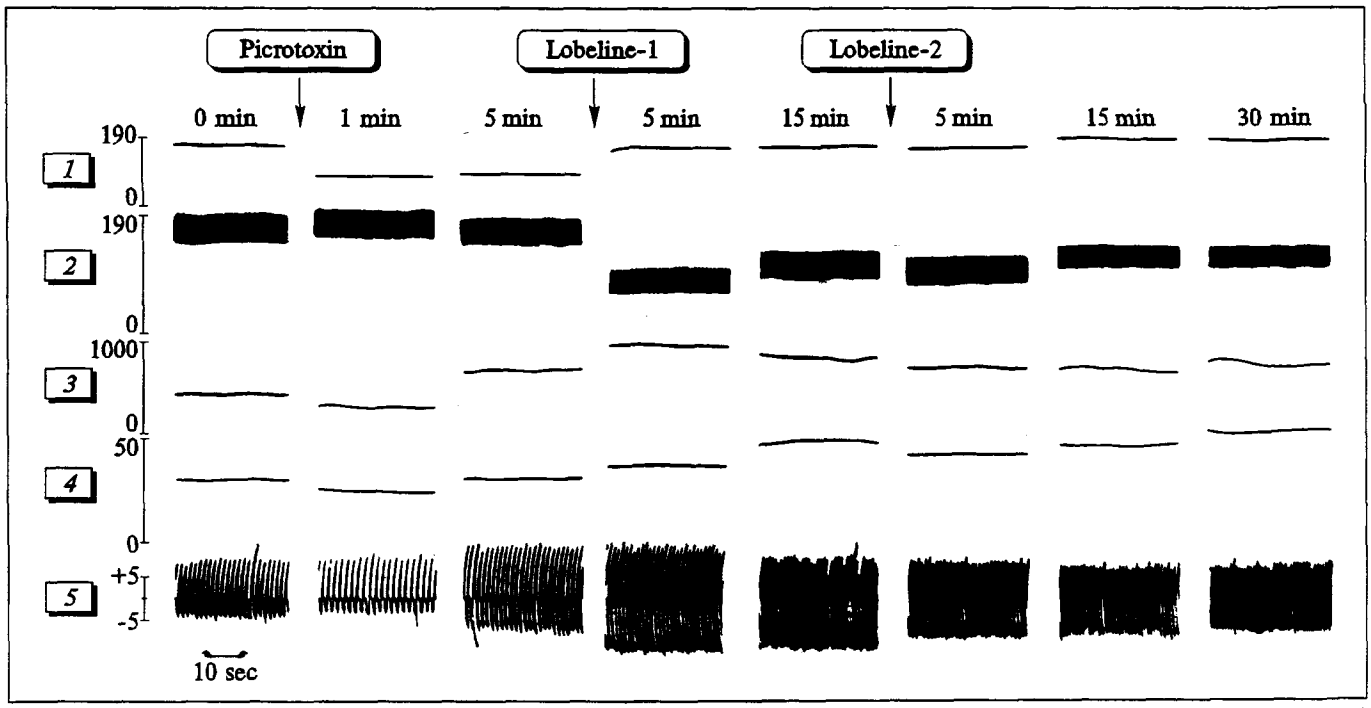

Fig. 2. Effect of two intraventricular lobeline injections (800 nmol/kg each) in a cat preinjected with picrotoxin (30 nmol/kg) by the same route. Here and in Fig. 3: *1*) heart rate, beats/min; *2*) arterial pressure, mm Hg; *3*) minute volume, ml/min; *4*) respiratory rate, min^{-1}; *5*) pneumotachogram, liters/min.

ade, it became evident that picrotoxin prevented a drop in respiratory rate and that the cessation of respiratory movements occurred as a result of a progressive decrease in the depth of respiration.

Comparison of our results on cats with those reported by other authors who studied the effect of lobeline on respiration in rats [11,12] suggests similar effects of lobeline on respiration in both species and a central action of this compound when it inhibits respiration in cats. The presence of N-cholinergic receptors in brainstem structures involved in the regulation of respiration [3,9] indicates that these receptors may be implicated in mediating the effects of lobeline and tubocurarine on respiration. As shown in a number of studies, the $GABA_A$ receptors located on the ventral sur-

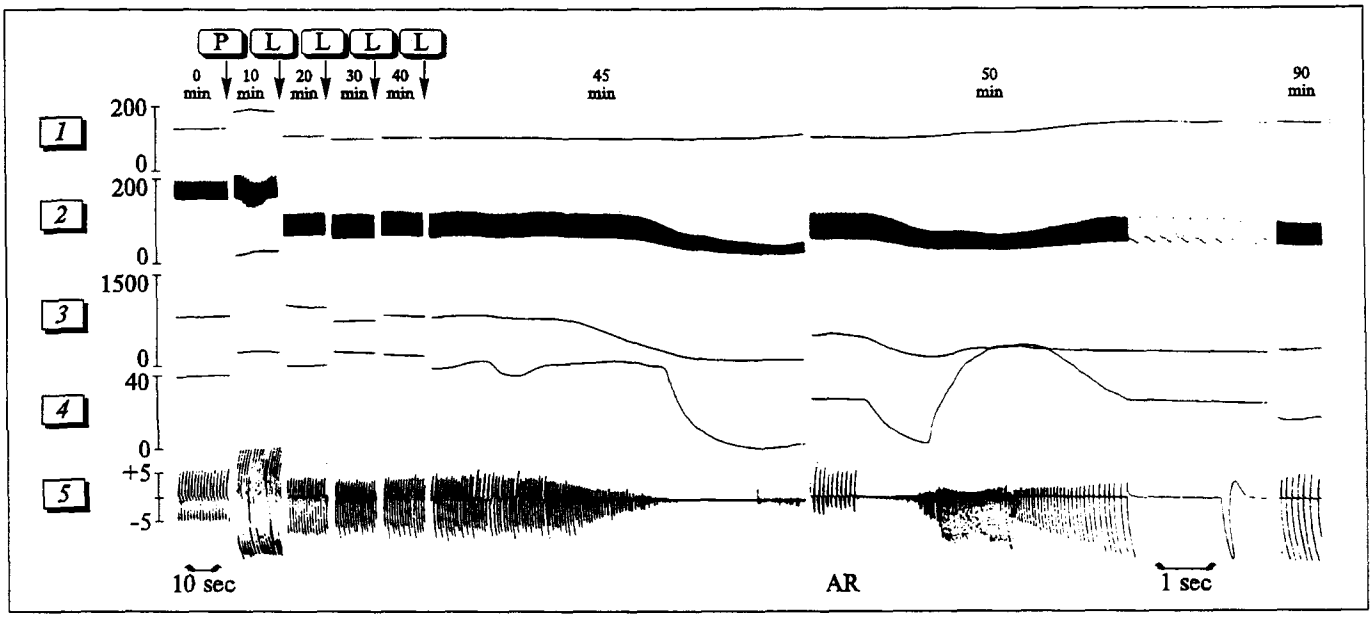

Fig. 3. Occurrence of gasping-like respiration in a cat injected repeatedly with lobeline by the intraventricular route (*L*, each dose 1.0 μmol/kg) after being preinjected with picrotoxin (*P*, 30 nmol/kg). Respiratory arrest occurred at minute 45, while gasping-like respiration lasted between minutes 50 and 90. *AR*: artificial respirator.

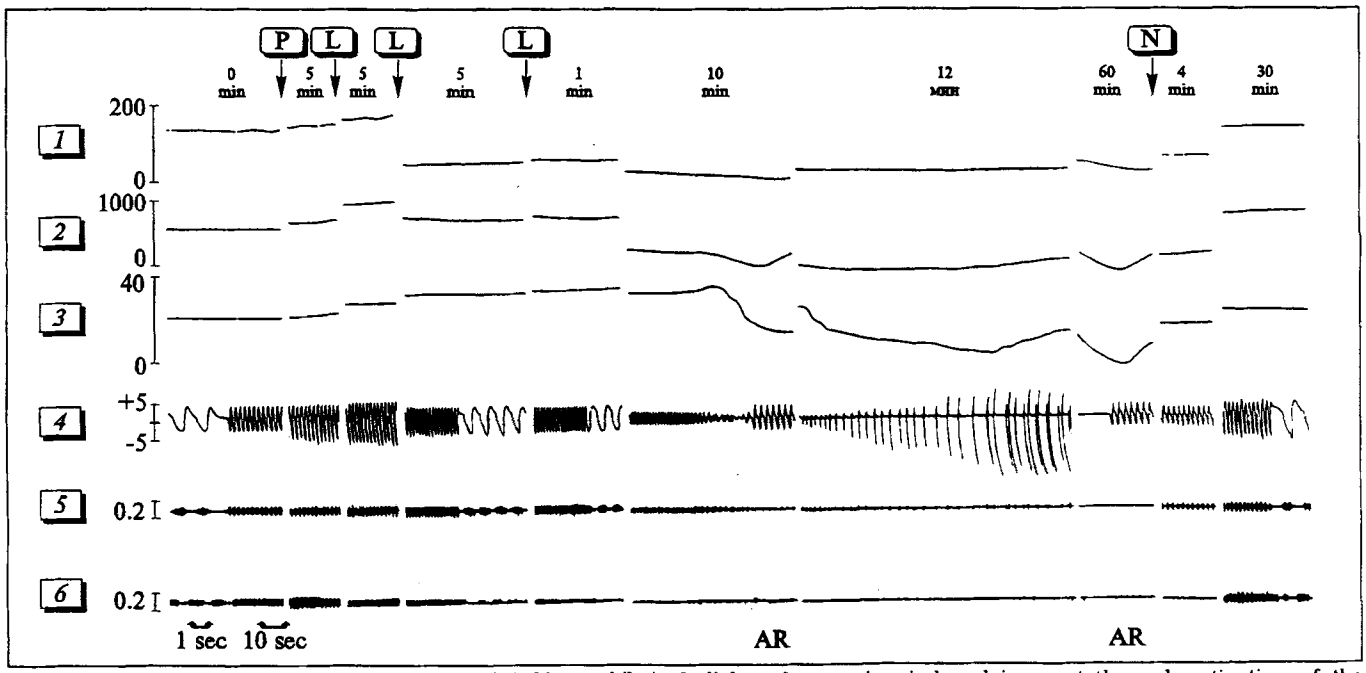

Fig. 4. Intraventricularly injected naloxone (*N*, 20 nmol/kg) abolishes the gasping induced in a cat through activation of the central N-cholinergic receptors with lobeline (*L*, each dose 1.0 μmol/kg) injected after picrotoxin (*P*, 30 nmol/kg). Gasping lasted between minutes 12 and 60 and was followed by respiratory arrest and naloxone administration. *1*) arterial pressure, mm Hg; *2*) minute volume, ml/min; *3*) respiratory rate, min^{-1}; *4*) pneumotachogram, liters/min; *5*) electromyogram of phrenic muscle, mV; *6*) electromyogram of internal intercostal muscle, mV. *AR*: artificial respirator.

face of the medulla oblongata and in some part of the posterior hypothalamus help regulate both respiratory volume and respiratory rate [5,7].

Taken together, the present results suggest that respiration may be inhibited following activation of the central N-cholinergic receptors by the GABA-ergic system and that the $GABA_A$ receptors are probably involved in slowing the respiratory rhythm.

Gasping-like respiration may result from a partial decrease in the influence of the GABA-ergic system ($GABA_A$ receptors) and a simultaneous increase in the activity of the central N-cholinergic receptors. It is significant that the respiratory abnormalities can then be eliminated by blocking the opioidergic system of the brain, which indicates that this system participates in the development of terminal respiration, and that naloxone may be used to correct respiratory disturbances of central genesis. There is evidence suggesting the participation of endogenous opioids in respiratory control and in deafferentation of central structures of the respiratory apparatus, which is the main feature of terminal respiration [1]. Moreover, one cause of terminal respiration of the gasping type may be acute hypoxia [1]. It cannot be ruled out that gasping in our experiments was also caused by the hypoxia resulting from a sharp and long-lasting decrease in blood pressure consequent to the administration of lobeline in the presence of picrotoxin. However, no gasping-like respiration was developed by any of the animals in which blood pressure drop was caused by lobeline alone.

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