MICROIONTOPHORETIC STUDY OF THE MECHANISM OF ACTION OF GAMMA-HYDROXYBUTYRIC ACID

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The effect of gamma-hydroxybutyric acid (GHBA) on extracellularly recorded spontaneous unit activity in the sensomotor cortex was studied by microiontophoresis in rabbits. GHBA reduced the discharge frequency of most neurons. Bicuculline, a specific blocker of gamma-aminobutyric acid (GABA), prevented the inhibitory effect of GHBA. This indicates that competitive relations may arise between GHBA and GABA while they interact with the common receptor. The conditions for the appearance of such relations are discussed.

KEY WORDS: gamma-hydroxybutyric acid; gamma-aminobutyric acid; bicuculline; microiontophoresis; sensomotor cortex; epilepsy.

Gamma-hydroxybutyric acid (GHBA) is a natural metabolite of the brain [7]. It is a compound similar in structure to gamma-aminobutyric acid (GABA), the hypothetical inhibitory mediator of the CNS. GHBA and its sodium salt (sodium hydroxybutyrate) pass readily through the blood—brain barrier and exert a sedative and hypnotic action [1, 3, 12]. In large doses sodium hydroxybutyrate possesses a narcotic effect. Because of these properties the compound has found wide clinical application.

The mechanism of the effect of GHBA on brain functions has not yet been finally settled. GHBA is known to cause accumulation of dopamine in the corpus striatum [9]. This feature is linked both with an increase in the synthesis of dopamine and a decrease in its liberation from presynaptic endings. This effect has been shown to be connected with inhibition of impulsation on dopaminergic nerve fibers [14]. It is logical to suppose that inhibition of impulsation of dopaminergic neurons is due to interaction between GHBA and the GABA receptors of central inhibitory synapses. In view of the absence of direct proof of this hypothesis, it was decided to investigate the possibility of competitive relations between GHBA and GABA during microiontophoretic application of the two substances to nerve cells.

METHODS

Experiments were carried out on 18 adult rabbits. The animals were immobilized with diplacin* (5 mg/kg) and artificially ventilated. Fixation points of the skull were infiltrated with the long-acting local anesthetic proloncain. Altogether, 86 sensomotor cortical neurons were studied. For extracellular recording of spontaneous unit activity and for microiontophoretic injection of the chemicals, 7-channel microelectrodes were used. The channels of the microelectrodes were filled with aqueous solutions of the following substances: NaCl (3M); GABA (0.5M, pH 3.0); GHBA (0.5M, pH 7.1); GHBA-Na (0.5M, pH 7.1); Na glutamate (0.3M, pH 7.5); bicuculline hydrochloride (0.005M, pH 3.0). GABA and bicuculline were injected by a current of positive polarity, the other agents by a current of negative polarity. The microiontophoretic currents were compensated with an error of ± 2 nA. Only those neurons whose action potentials were at least 1 mV were taken into account. Other details of the method were described previously [2, 10].

RESULTS

GHBA, injected microiontophoretically, reduced the frequency of action potentials generated by most neurons tested (55%). The degree of its inhibition increased with an increase

*1,3-di(β-platyneciniumethoxy)benzene hydrochloride.

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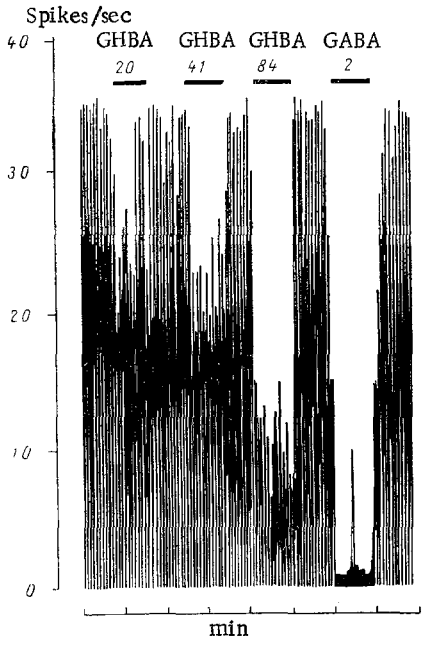

Fig. 1. Inhibitory action of GHBA and GABA on spontaneous unit activity in the rabbit sensomotor cortex. Abscissa, time (in min); ordinate, discharge frequency of neuron. GHBA) Gamma-hydroxybutyric acid; GABA) gamma-aminobutyric acid. Horizontal lines show duration of application of agents, numbers above them show intensity of microiontophoretic currents in nA ($1 \cdot 10^{-9}$ A). Remainder of explanation in text.

in the strength of the applying current, i.e., with an increase in the dose of the injected agent (Fig. 1). The inhibitory effect of GHBA was always less than the depression induced by injection of GABA into the region of the same neurons. As Fig. 1 shows, GABA applied by a current of 2 nA caused complete suppression of unit activity, whereas GHBA, applied by a much larger current (84 nA), had a less marked inhibitory effect. On the other hand, GHBA did not change the spontaneous discharge frequency of 33% of the neurons tested. GABA usually had a strong inhibitory action on these neurons.

To discover whether inhibition induced by GHBA is the result of specific interaction between the agent and the GABA-receptors of the chemoreceptive membrane of the neurons, it was necessary to study the effect of bicuculline, which blocks GABA receptors, on the action of GHBA. It will be clear from Fig. 2 that GHBA, applied to the neuron membrane by a current of 92 nA, caused complete suppression of unit activity. This suppression was evidently not connected with any local anesthetic (membrane-stabilizing) action of GHBA, for it was easily overcome by application of glutamate by a small current (5 nA). Microiontophoretic application of bicuculline against the background of continued application of GHBA blocked the inhibitory effect of the latter. These results were obtained by a study of four neurons. In two of six neurons of this series no antagonism was found between bicuculline and GHBA.

Finally, an increase in the frequency of spontaneous activity under the influence of GHBA was found in 12% of neurons investigated. An example of this effect of GHBA is given in Fig. 3. Clearly, by contrast to the inhibitory effect of GABA, GHBA had an excitatory action on the same neuron and increased its discharge frequency. Furthermore, the inhibitory action of GABA, if applied against the background of continued application of GHBA, was much weaker than initially and it was restored only when the current applying GHBA was removed. It should be especially emphasized that this excitatory effect of GHBA, opposing the inhibitory action of GABA, was observed mainly in neurons discharging volleys or bursts of high-frequency activity. Half of the neurons excited by GHBA belonged to animals which had developed traumatic epilepsy.

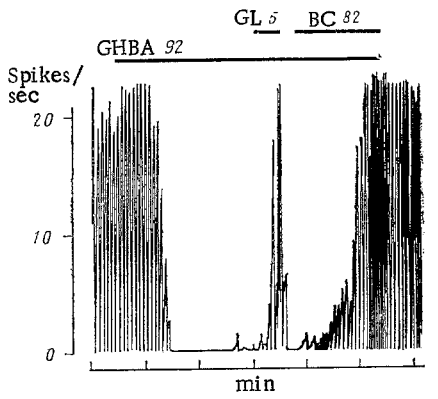

Fig. 2. Blocking inhibitory effect of GHBA by bicuculline. GL) Sodium glutamate, BC) bicuculline. Remainder of legend as in Fig. 1.

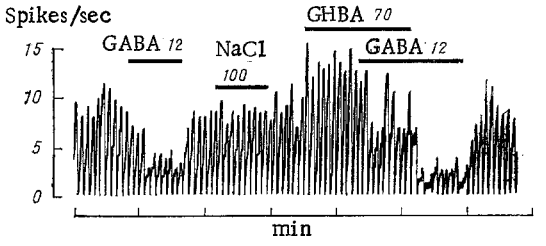

Fig. 3. Excitatory action of GHBA on unit activity. Legend as in Fig. 1.

GHBA-Na in these experiments always modified unit activity in a similar manner to GHBA itself.

It can be concluded from the experimental results described above that GHBA, like GABA, reduces the frequency of spontaneous activity of most sensomotor cortical neurons. The failure of other workers [8] to find an inhibitory effect of GHBA during microiontophoretic application of the agents to cortical neurons can perhaps be explained on the grounds that GHBA, unlike many other agents, exerts its action only when the tip of the microelectrode is as close as possible to the neuron membrane. In the present experiments withdrawal of the microelectrode even a very small distance from the cell led to disappearance of the GHBA effect. This may be connected both with the low activity of GHBA and (or) with the high activity of the tissue mechanisms for its inactivation.

Blocking of the inhibitory effect of GHBA by bicuculline indicates that GABA receptors may participate in the mechanism of action of GHBA. These observations are in agreement with results obtained by Roth and Nowycky [13], who showed that picrotoxin, another blocker of GABA receptors, blocks the accumulation of catecholamines in the corpus striatum induced by GHBA.

The excitatory action of GHBA, opposite to the inhibitory effect of GABA, discovered in some neurons shows that competitive relations may arise between two agents during their interaction with the common receptor. This competition is most likely when GABA-ergic inhibitory control over unit activity is strengthened. For example, in epilepsy high-frequency discharges of a neuron can cause self-limitation of its own discharge frequency through the activation of inhibitory mechanisms (recurrent inhibition and so on). Cortical inhibition, in the generally accepted view, is GABA-ergic [11]. GHBA, applied to such neurons, binds with GABA receptors and, because of its low intrinsic activity, cannot replace GABA in its physiological action. GHBA thus behaves as a competitive antagonist of GABA and causes "disinhibition" i.e., excitation, of nerve cells.

Antagonism between GHBA-Na and GABA was first found by Uspenskii and Listvina [5], who studied the effect of these substances on behavior in chickens. They attributed this antagonism to competition between GHBA and GABA for GABA-transaminase. Ostrovskaya and Schmidt [4], who investigated recovery cycles of primary evoked responses in the rabbit cortex, also found that sodium hydroxybutyrate has a GABA-lytic action and they postulated competition between hydroxybutyrate and GABA for GABA receptors.

Antagonism between hydroxybutyrate and GABA may lie at the basis of the motor excitation and even conclusions observed by different workers during the experimental and clinical study of sodium hydroxybutyrate [3, 6, 15].

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