**Benzodiazepines are synergic with γ-aminobutyric acid. Microiontophoretic evidence**

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**INTRODUCTION**

Numerous literature data indicates that benzodiazepines influence neuronal activity in various sections of central nervous system, from spinal cord to the brain stem up to neocortex.

It was shown that benzodiazepines increase the presynaptic inhibition in the spinal cord [1-3] and various types of postsynaptic inhibition in the cerebellum [4] and nigrostriatal system [5]. Since these types of inhibition seem to be mediated by γ-aminobutyric acid (GABA) [6], an assumption was made as to the influence of benzodiazepines on GABA-ergic synaptic transmission [5, 7].

However, from the works by Steiner and Felix [8] and Gähwiler [9] carried out on cerebellum and Deiters nucleus neurons, it seems that benzodiazepines not only fail to potentiate central inhibitory processes but even hamper them, being themselves GABA antagonists. In spite of recent polemics [10], so far the above mentioned contradiction has not been settled [11].

We studied the interaction of chlordiazepoxide (CDP) with GABA during simultaneous microiontophoretic application of both agents to the neurons of rabbit sensorimotor cortex. GABA seems one of the most likely inhibitor transmitters in the neocortex [6]. The influence of the systemically administered diazepam on GABA-ergic inhibitory processes in the cerebral cortex was studied in previous investigations [12, 13].

**MATERIALS AND METHODS**

The experiments were carried out on 9 adult rabbits weighing 3-4 kg. Under hexobarbital sodium narcosis the cortical surface was laid bare. The animals were then curarized with gallamine and submitted to artificial
respiration. The wound was infiltrated with a long acting local anaesthetic.

Microiontophoretic application of the substances and extracellular registration of the neuronal electrical activity were carried out by using 5-7 barrelled microelectrodes prepared according to a method described earlier [14]. Central recording barrel was filled with 2M NaCl. Lateral barrels contained the following substances: chlordiazepoxide hydrochloride (0.2 M; pH = 3.0), GABA (1M; pH = 3.0), sodium glutamate (1M; pH = 7.5) and 3M NaCl. The influence of the current on the neuronal activity was minimized using standard current balancing techniques. Retaining currents were between 15-20 nA.

RESULTS

In Fig. 1 is shown a typical influence of microiontophoretically applied CDP on the spontaneous activity of a neuron of sensorimotor cortex, which consists of a decrease of the frequency of spontaneous activity. The inhibiting effect of CDP increased with the increase of the ejecting current and thus

![Graph showing dose-dependent inhibitory action of microiontophoretically applied chlordiazepoxide (CDP) on the glutamate-evoked firing of a neuron of the sensorimotor cortex in the rabbit. The applications of agents are indicated by horizontal bars, the figures above them indicate currents in nA (1 × 10^-9A). Glutamate was applied by 10 nA current. Rate of firing is displayed by an integrated rate meter calibrated on ordinate in spikes/sec. The rate meter was reset every 3 sec.]
of the dose of the agent applied to the cell. Control application of the Na+ ions failed to induce any significant change in the cellular activity. The depressing dose-dependent CDP action was observed in all the 29 analysed neurons. In no cases CDP did facilitate neuronal activity.

Fig. 2 shows the results of the simultaneous microiontophoretic application of CDP and GABA to the neuron. In Fig. 2 a GABA, applied by 4 nA current, induced an almost complete inhibition of the neuronal activity. This effect of GABA is still present if the drug is ejected during a long-term CDP application from another barrel of the microelectrode.

Fig. 2 b shows the opposite situation. When CDP is applied during neuronal depression caused by GABA, does not prevent GABA inhibitory effect. On the contrary, a potentiation of the effect of the aminoacid was observed.

During simultaneous CDP and GABA application, an additive depressing effects of the agents was observed. To obtain such an effect it was necessary to select submaximal doses of CDP and GABA, inducing only a slight inhibition of the neuronal spontaneous activity. Such an additive effect is demonstrated in Fig. 3. GABA injected by a 2 nA current caused a decrease of the frequency of spontaneous neuronal activity equal approximately to 50 % of the initial frequency of spontaneous activity. CDP applied by a 6 nA current induced an inhibition of spontaneous activity of approximately 25 %. Joint GABA and CDP application (in the same doses) resulted in a depressing effect equal to the sum of the effects of GABA and CDP (about 75 %).

In two experiments urethane and chloralose were used for narcosis. The results of these experiments did not differ from those obtained with hexobarbital sodium narcosis.

DISCUSSION

Our data did not confirm the results of Steiner and Felix [8] and of Gähwiler [9] on the antagonism between benzodiazepines and GABA.

Other authors also failed to find an antagonism between CDP and GABA during microiontophoretic application of the drugs to brain stem [15], spinal cord and cerebellar neurons [16]. In these studies, it was found that microiontophoretic injection of benzodiazepines and GABA induce depression and also that the GABA-blocker bicuculline is able to eliminate the effects of benzodiazepines [15]. This antagonism between diazepam and bicuculline has been confirmed in our laboratory [17].

At present, the reasons for the divergence between our data and the results obtained by Steiner and Felix are not yet clear. It is possible that
Fig. 2. — Lack of antagonism between CDP and GABA applied microiontophoretically to the same neuron:

a) Effect of GABA on the background of long-term CDP application.

b) Effect of CDP on the background of long-term GABA application (another neuron).

The activity of both neurons was maintained by the application of glutamate by:

a) 3 nA;

b) 7 nA.

Rate meter was reset every 3 sec in a) and every 2.5 sec in b). See Fig. 1 and the text for other explanations.
certain differences in the conditions of the experiment could lead to quite opposite effects.

From the data obtained by Gähwiler [9] on Purkinje cells of explants of rat cerebellum it results that in low doses benzodiazepines facilitate electrical activity of the neurons and prevent GABA inhibitory effect. In high doses they do not antagonize GABA and inhibit neuronal activity. Thus it could be assumed that Steiner and Felix applied microiontophoretically to receptors smaller quantities of chlordiazepoxide than we did.

The correlation of the concentrations of the agents under investigation seems therefore to be a considerable value. It is known that there may exist between two substances active on the same receptor not only synergistic relationships but also — at certain concentrations — antagonistic, competitive relationships. Such an effect is typical of substances possessing great affinity with the receptor and small intrinsic activity [18]. As it was pointed out above, CDP is several times less active than GABA in inducing neuronal depression.

On the other hand, in earlier experiments with systemic diazepam administration, we found a potentiation of the inhibitory effects of micro-
iontophoretically applied GABA [13, 17]. However, Steiner and Felix, upon systemic administration of similar doses, found an antagonism between benzodiazepines and GABA.

The observed potentiation of the depressing effects of microiontophoretically applied GABA after intravenous diazepam administration can be explained with an increase in the sensitivity of cortical GABA-receptors induced by benzodiazepines [13]. However, according to Squires and Brac­strup [19] specific nervous tissue receptors are not the same for benzodiazepines and for GABA.

The hypothesis of a direct influence of benzodiazepines on the GABAergic inhibitory synaptic transmission requires further confirmation and specification. One should remember the possibility that the benzodiazepines could interfere with other brain mediatory system: cholinergic, monoaminergic, as pointed out by Straughan [11].

Summary. — By means of microiontophoresis chlordiazepoxide (CDP) was applied to the neurons of rabbit sensorimotor cortex. CDP decreased the frequency of spontaneous electrical activity of all the neurons studied in a dose-dependent manner. During simultaneous CDP and γ-aminobutyric acid (GABA) application an additive effect of the agents was observed. No antagonism between CDP and GABA was found. Probable reasons for contradictory data obtained by different AA dealing with benzodiazepine effect on GABA-ergic inhibitory processes are discussed.

REFERENCES


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