leads to a corresponding decrease in their inhibitory effect on dopaminergic nigrostriatal neurons and, consequently, to activation of the dopamine turnover [8].

Consequently, it can be concluded from the results of this investigation that the most effective drugs for abolishing the sleep disorders in the alcohol withdrawal period are sodium hydroxybutyrate, which restores physiologically normal sleep, and phenazepam, which has a powerful sedative and hypnotic effect. Administration of apomorphine leads to a tendency toward the normalization of sleep although normal levels of sleep indices are not achieved. Haloperidol is probably unsuitable for correcting sleep disorders during the period of alcohol withdrawal.

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NIPECOTIC ACID, A COMPETITIVE INHIBITOR OF ^{3}H-GABA NET UPTAKE BY RAT BRAIN SYNAPTOSOMES

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Nipecotic acid (piperidine-3-carboxylic acid) is known as an active inhibitor of the ^{3}H-GABA uptake system, with a high degree of affinity [1, 7]. It has been shown that nipecotic acid and GABA use the same carrier, and that the former has a higher degree of affinity for it than GABA [6]. It is generally considered that inhibition of GABA uptake by nipecotic acid takes place by a noncompetitive mechanism [5]. Recently the idea has been developed that homoexchange of GABA takes place at the level of the presynaptic terminal, and that it may play a role in the accumulation of the labeled exogenous amino acid [8, 9], and new techniques have been developed [10] for isolating the so-called pure or net uptake of GABA. These developments have provided a basis for the comparative study of the kinetics of inhibition by nipecotic acid of the "apparent" and net uptake of ^{3}H-GABA by synaptosomes isolated from the rat cerebral cortex, and the investigation described below was devoted to this problem.

EXPERIMENTAL METHOD

Synaptosomes were isolated from the rat cerebral cortex by Hajos' method [4]. Half of the material obtained was not subjected to further treatment, and the other half was used to prepare "depolarized" synaptosomes. For this purpose the synaptosomes were suspended in medium with an increased K^+ concentration

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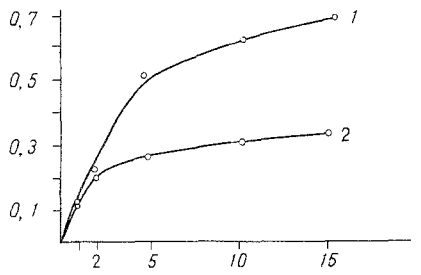

Fig. 1

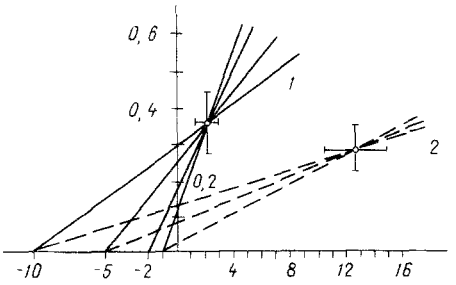

Fig. 2

Fig. 1. Effect of concentration of ^{3}H-GABA on rate of its accumulation by synaptosomes. 1, 2) ^{3}H-GABA uptake by "ordinary" and depolarized synaptosomes respectively. Abscissa, ^{3}H-GABA concentration (in μM); ordinate, accumulation of ^{3}H-GABA (in nmoles/3 min/mg protein).

Fig. 2. Kinetics of inhibition by nipecotic acid of net ^{3}H-GABA uptake by synaptosomes. 1) Control, 2) 20 μM nipecotic acid. Abscissa, concentration of ^{3}H-GABA in medium (in μM); ordinate, rate of accumulation of ^{3}H-GABA (in nmoles/min/mg protein).

(NaCl 100 mM, KCl 56 mM, $CaCl_2$ 1 mM, glucose 10 mM, sucrose 100 mM in 30 mM Tris-phosphate buffer, pH 7.4). The resulting suspension was incubated in depolarizing medium for 10 min at 37°C, after which the synaptosomes were sedimented by centrifugation (18,000g, 10 min), and the residues were suspended in 0.32 M sucrose. The subsequent study of the kinetics of ^{3}H-GABA uptake by both types of synaptosomes was carried out under identical conditions, fully described previously [3]. Incubation of 50 μl of the synaptosomal suspension was carried out in medium with a physiological K^+ concentration (5 mM), with continuous shaking. The process was stopped after 3 min by rapid ultrafiltration on a Millipore apparatus, using membrane filters with a nominal pore size of 0.45 μm. The samples were applied to the filter in a volume of 0.2 ml and washed with 15 ml 0.32 M sucrose. The concentration of labeled mediator on the filter was determined by liquid scintillation spectrometry (Intertechnique SL-4000 counter). The results of six to nine measurements were subjected to statistical analysis with calculation of the mean values and their confidence intervals at the P = 0.05 level, and displayed in Eisenthal–Cornish-Bowden rectilinear kinetic coordinates [2].

EXPERIMENTAL RESULTS

The results of the study of the kinetics of the inhibitory effect of nipecotic acid on ^{3}H-GABA uptake by ordinary synaptosomes in the present experiments were in good agreement with data in the literature [5]. For instance, inhibition was noncompetitive in type, with no change in the Michaelis constant (K_m), which was 5.8 ± 1.6 μM (in control samples without addition of the inhibitor K_m = 5.5 ± 1.2 μM). The maximal reaction velocity was reduced from 0.97 ± 0.10 nmole/min/mg protein in the control to 0.40 ± 0.08 nmole/min/mg protein. Calculation of the inhibition constant (K_i) gave the value 13 ± 3 μM, also in agreement with the published data [5].

Meanwhile it is known that K_m for nipecotic acid as an inhibitor of GABA uptake is equivalent to the value of K_m for the uptake of nipecotic acid itself [7]. Uptake of the latter also is inhibited by GABA, and its liberation is stimulated by GABA. Both these effects also are observed in an inverse relationship [6]. From a consideration of all these facts it appears that, on the one hand, GABA and nipecotic acid can use the same mobile carrier [12], and on the other hand, that the noncompetitive type of inhibition of uptake observed by ourselves and other workers is contradicted [5]. A similar disagreement in the case of another inhibitor (L-2,4-diaminobutyric acid) has been interpreted [11] as the result of time differences in the order of addition of the substrate and blocker to the incubation medium, as a result of which inhibition of competitive type takes place on the outer surface of the synaptosomal membrane (simultaneous addition of substrate and inhibitor to the medium) and of the noncompetitive type on the inner surface of the membrane (preincubation with inhibitor).

Considering the complex nature of the phenomena observed, it was assumed that homoexchange of ^{3}H-GABA plays a considerable role in the process of its accumulation by synaptosomes [9]: according to this view the kinetic parameters of net transport can only be correct if allowance is made for the participation of homoexchange.

To study homoexchange, the technical recommendations based on potassium depolarization of synaptosomes before incubation with the label were followed [10]. For this purpose ordinary synaptosomes were subjected to preliminary depolarization. The results of the study of dependence of "apparent" and net uptake on the GABA concentration are given in Fig. 1, from which it follows that, if the ^{3}H-GABA concentration in the medium did not exceed 2 μM the curves for "apparent" and net uptake coincided. Starting from 4-5 μM net uptake flattens out on a plateau, whereas "apparent" uptake does not reach saturation between the concentration limits studied. After incubation for 3 min, with ^{3}H-GABA in a concentration of 5 μM, net uptake amounted to about 50% of the "apparent" value.

The results of the study of the kinetics of inhibition of net GABA uptake on the model of depolarized synaptosomes are given in Fig. 2. They show that values of K_m of 2.0 ± 0.9 μM and of the rate of accumulation of ^{3}H-GABA (V_{max}) of 0.38 ± 0.09 nmoles/min/mg protein, obtained in the control, differed from the corresponding values obtained in experiments with "ordinary" synaptosomes – 5.5 ± 1.2 μM and 0.97 ± 0.10 nmole/min/mg protein respectively.

In the presence of inhibitor the value of K_m was increased compared with the corresponding control up to 12.8 ± 3.1 μM, whereas the value of V_{max} was virtually unchanged at 0.29 ± 0.06 nmole/min/mg protein. These results are clear evidence in support of the competitive nature of inhibition. The value of K_i calculated from the data of these experiments was 3.9 ± 0.2 μM.

It can thus be concluded that the process of ^{3}H-GABA accumulation is complex in nature and takes place in vitro with the participation of at least two mechanisms – homoexchange and net uptake of mediator. The competitive character of inhibition of net uptake by nipecotic acid and the noncompetitive nature in the case of "apparent" accumulation of GABA are evidence that when the kinetic parameters of inhibition of presynaptic GABA transport are determined the important contribution of homoexchange of the amino acid to this process must be borne in mind. Meanwhile the results confirm the existence of net GABA uptake.

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