action on the sodium current can be explained (by analogy with the action of catecholamines and their derivatives) by their nonspecific binding with membrane lipids [8].

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IONIC DEPENDENCE OF GABA-POTENTIATING EFFECTS OF BENZODIAZEPINE TRANQUILIZERS AND HARMAN

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Postsynaptic responses of central neurons evoked by gamma-aminobutyric acid (GABA) are potentiated by benzodiazepine tranquilizers [1, 8, 12] and by harman, an endogenous ligand of benzodiazepine receptors [5]. The GABA-potentiating action of chlordiazepoxide and harman is similarly dependent on chloride concentration in the medium [1, 5], but the GABA-potentiating action of chlordiazepoxide is exhibited over the concentration range 10^{-7}-10^{-5} M. With a further increase in the harman concentration the GABA-potentiating effect weakens as the depolarizing action characteristic of harman, which is connected with blockade of the electrically excitable K^+ channels [5], develops.

Considering that the slow outward potassium and inward calcium electrically excitable currents in spinal ganglionic neurons of young rats have a similar time course [6] and the work pattern of receptor-linked ionic channels of chemically excitable membranes, besides other factors, depends essentially on the intracellular Ca^{++} ion concentration [3], it was decided to investigate whether a causal connection exists between the ability of harman to block electrically excitable K^+-channels and its GABA-potentiating activity. With this aim, the dependence of the GABA-potentiating effects of harman and chlordiazepoxide on the external Ca^{++} ion concentration and their changes under the influence of blockers of electrically excitable K^+- and Ca^{++}-ionic channels were compared.

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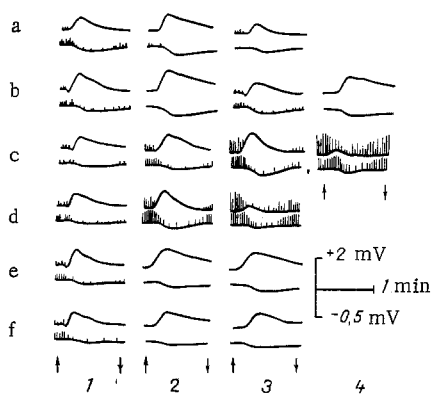

Fig. 1

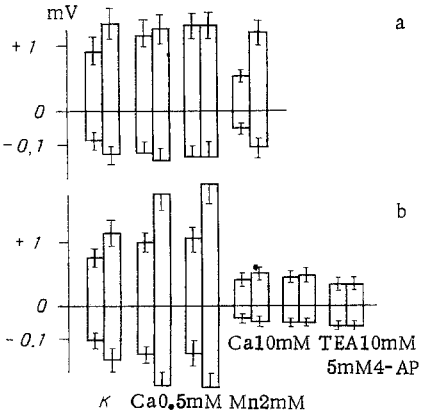

Fig. 2

Fig. 1. Effect of change in ionic medium and of blockers of electrically excitable channels on GABA effects in experiments on isolated rat spinal cord. Electronic dorsal (top traces in each pair) or ventral (bottom traces) root potentials evoked by GABA ($1 \cdot 10^{-4}$ M) during superfusion of spinal cord with solution of standard ionic composition (1 and 3b) and with various Ca^{++} concentrations in superfusing solution (2a 0.5 mM, 3a 10 mM) in the presence of 2 mM Mn^{++} ions (2b, e2, f2) or Co^{++} ions (b4), 4-aminopyridine (2c 0.01 mM, 3c 0.1 mM, 4c 5 mM), TEA (2d 0.5 mM, 3d 10 mM), 0.1 mM 4-aminopyridine and 2 mM Mn^{++} ions (3c) or 0.5 mM TEA and 2 mM Mn^{++} ions (3f). Arrows indicate beginning and end of superfusion of spinal cord with solution containing GABA. Upward deviation of curve corresponds to negativity beneath proximal electrode.

Fig. 2. Changes in GABA-potentiating action of harman (a) and chlordiazepoxide (b) with different Ca^{++} concentrations in medium and against the background of blockers of electrically excitable Ca^{++}- (2 mM Mn^{++} ions) and K^+-channels (10 mM TEA and 5 mM AP). Top columns show responses of primary afferents, bottom columns – responses of motoneurons to GABA. Left column of each pair denotes initial response, right column shows effect of harman or chlordiazepoxide in a concentration of $1 \cdot 10^{-5}$ M.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated spinal cord of young rats aged 8-15 days. Electrotonic dorsal and ventral root potentials evoked by application of GABA ($1 \cdot 10^{-4}$ M) to the spinal cord were recorded by the method described previously [2].

In the experiments of series I electronic ventral and dorsal root potentials evoked by GABA were studied during variations in the external Ca^{++} concentration (0.5, 2.5, and 10 mM), and during the action of blockers of electrically excitable calcium (Mn^{++} and Co^{++} ions, 2 mM) or potassium channels [tetraethylammonium (TEA), 0.5 and 10 mM; 4-aminopyridine, 0.01, 0.1, and 5 mM]. Superfusion of the spinal cord with solutions containing a modified concentration of Ca^{++} ions and with addition of blockers of electrically excitable ionic channels continued for 20 min.

In the experiments of series II the GABA-potentiating action of the benzodiazepine tranquilizer chlordiazepoxide ($1 \cdot 10^{-5}$ M) was recorded with different concentrations of Ca^{++} ions in the solution surrounding the spinal cord, and during blockade of electrically excitable K^+- or Ca^{++}-channels of the spinal neuron membranes by TEA (10 mM), 4-aminopyridine (5 mM), or Mn^{++} ions (2 mM) respectively.

In the experiments of series III the GABA-potentiating action of harman ($1 \cdot 10^{-5}$ M with different Ca^{++} concentrations in the medium and in the absence and presence of Mn^{++} (2 mM) was investigated.

The experimental results were subjected to statistical analysis by the usual method.

EXPERIMENTAL RESULTS

Primary afferent depolarization and hyperpolarization of motoneurons of the isolated rat spinal cord, due to GABA, depend on the Ca^{++} concentration in the medium. An increase in the Ca^{++} concentration in the solution from 2.5 to 10 mM reduces responses of the neurons to GABA and potentiates GABA-induced desensitization of the neurons (Fig. 1). Conversely, when the Ca^{++} concentration in the medium fell to 0.5 mM, the effects of GABA increased but the degree of desensitization decreased (Fig. 1). Blockers of electrically excitable Ca^{++}-channels (Mn^{++} and Co^{++} ions), which potentiate GABA-induced responses of neurons and reduce their de-

sensitization, had a similar action. It can be tentatively suggested that GABA-potentiating effects of low concentrations of Ca^{++} ions in the medium, and also of Mn^{++} and Co^{++} ions, are connected with a fall in the intracellular Ca^{++} ion concentration and a change in the work pattern of chloride channels coupled with GABA receptors (an increase in the mean duration of their open state). Enhancement of chloride-dependent responses of acetylcholine when Mn^{++} and La^{+++} ions were present in the external solution, or with a fall in the intracellular Ca^{++} concentration, has been observed on dialyzed neurons of Limnea stagnalis [4]. Experiments on frog neuromuscular synapses showed a decrease by half in the mean duration of the open state of the ionic channels coupled with acetylcholine receptors, when the nerve–muscle preparation was kept in isotonic calcium chloride solution [3].

Classical blockers of electrically excitable K^+-channels, namely TEA (0.5 mM) and 4-aminopyridine (0.01 and 0.1 mM), also potentiated responses of primary afferents and motoneurons to GABA (Fig. 1), mediated through a calcium-dependent mechanism. An increase in the TEA and 4-aminopyridine (AP) concentration to 10 and 5 mM respectively depressed responses of primary afferents and motoneurons to GABA (Fig. 1). Weakening of the effects of GABA and potentiation of desensitization by high concentrations of blockers of electrically excitable K^+-channels were probably due to an increase in the intraneuronal Ca^{++} concentration. The GABA-potentiating effects of chloridizepoxide and harman were also found to be dependent on the functional state of the electrically excitable Ca^{++}- and K^+-channels.

During the action of a solution containing 10 mM Ca^{++} ions on the spinal cord the GABA-potentiating action of chlordiazepoxide was depressed by a greater degree than responses of the neurons to GABA (Fig. 2). A similar result was observed during the action of blockers of electrically excited K^+-channels: TEA (10 mM) and AP (5 mM). On the other hand, GABA-potentiating action of chlordiazepide was enhanced in medium deficient in Ca^{++} (0.5 mM) and in the presence of Mn^{++} ions (Fig. 2). These facts are evidence that one of the probable mechanisms of the GABA-potentiating action of chlordiazepoxide may be a fall in the intracellular Ca^{++} concentration, leading to an increase in the mean duration of the open state of the chloride channels coupled with GABA receptors. The ability of benzodiazepines to increase the duration of the open state of these channels has been demonstrated by fluctuation analysis [10, 11]. If benzodiazepines do in fact lower the intracellular Ca^{++} concentration in neurons, they do so in a different way from the analogous effect of blockers of Ca^{++}-channels (Mn^{++} or Co^{++}), for unlike the latter benzodiazepines do not disturb synaptic transmission in the spinal cord [1].

The GABA-potentiating action of harman in medium deficient in Ca^{++} ions or in the presence of Mn^{++} ions, on the other hand, is reduced and enhanced in solution with excess of Ca^{++} ions (Fig. 2). In other words, the GABA-potentiating effect of harman is also calcium-dependent, but this dependence differs from the Ca^{++}-dependence of the GABA-potentiating effect of chlordiazepoxide. On the other hand, the character of dependence of the GABA-potentiating action of harman and the GABA-potentiating effect of classical blockers of K^+-channels (TEA or AP) on the Ca^{++} concentration in the medium correspond completely. The similarity between the action of harman and of blockers of electrically excitable K^+-channels is also confirmed by the fact that, like TEA and AP, harman in concentrations above 10^{-2} M weakens responses of neurons to GABA. These facts suggest the existence of a causal connection between the GABA-potentiating action of harman and its ability to block potential-dependent K^+-channels of nerve cells.

Judging from the directly opposite influences of different concentrations of Ca^{++} ions in the medium and blockers of electrically excitable calcium channels on GABA-potentiating effects of chlordiazepoxide and harman, the GABA-potentiating action of these substances is not based on identical mechanisms, although these substances interact with high affinity with "benzodiazepine receptors" [9].

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ANALYSIS OF THE MEMBRANE EFFECTS ON GANGLIOSIDES

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One piece of evidence confirming the validity of the concept of neurochemical control over the cerebral circulation [5] was the discovery that gangliosides possess cerebral vasoactivity [6]. It has been shown that an essential role in the effects of gangliosides on the cerebral vessels is played by their interaction with membrane structures [7].

The aim of this investigation was to study the effect of gangliosides on structural lability of the membranes.

EXPERIMENTAL METHOD

Fluorescence spectra were recorded on the MPF-2A spectrofluorometer (Hitachi, Japan) and absorption spectra on the VSU2-P spectrophotometer. If the optical density of the samples was over 0.1, the internal filter effect [9] was taken into account. The quantum yield of fluorescence was measured by a relative method [14] and shifts of the maximum of fluorescence by a two-wave method [10]. The concentration of the free and bound probe was found by calculation [4]. The NO_3^- ion was used as quencher of fluorescence. Accessibility of tryptophanyl residues for quenching was calculated by the method in [2], and the efficiency of energy migration by the method in [1]. Hill's coefficient was determined as the tangent of the angle of slope between Atkinson's coordinates. The total ganglioside fraction was isolated from brain tissue by the method in [12]. Purity of the gangliosides was verified by thin-layer chromatography. Human serum albumin (HSA) from Reanal (Hungary), liposomes obtained from egg lecithin [11], and erythrocyte membranes isolated from fresh donor's blood [13], were used.

EXPERIMENTAL RESULTS

Gangliosides in the initial period of interaction were shown to cause some degree of quenching of fluorescence of 1-anilinonaphthalene-8-sulfonate (ANS), bound with HSA. Later inversion of the effect was observed, to reach a maximum at the 30th minute of contact (Fig. 1a). Quenching of fluorescence of tryptophan residues of HSA by gangliosides depended on the incubation time and the ganglioside concentration (Fig. 1b). A graph of this relationship, plotted between Stern–Volmer coordinates, was concave in form, evidence of cooperativeness

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TABLE 1. Changes in Efficiency of Energy Migration under the Influence of Gangliosides (M ± m)

Donor	Acceptor	Control	Gangliosides	
			$2 \cdot 10^{-4}$ mM	$4 \cdot 10^{-4}$ mM
Tryptophan	ANS	0,31 ± 0,02	0,39 ± 0,02	0,37 ± 0,03
Tyrosine	Tryptophan	0,21 ± 0,02	0,26 ± 0,03	0,26 ± 0,02

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