Not withstanding the undoubted similarity to sodium hydroxybutyrate and lithium chloride [6], lithium hydroxybutyrate thus possesses definite specificty of action on the electroencephalographic effects of amphetamine, and this may perhaps be due to the special character of its pharmacokientics [11]. In the light of modern views on the role of the frontal cortex, amygdala, hippocampus [9], and caudate nucleus [14] in the genesis of affective disorders, the experimental data described above are in good agreement with recommendations that lithium hydroxybutyrate be used mainly in cyclic psychoses with a schizophrenia-like symptomatology [1].

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DISTURBANCE OF ^{3}H-GABA TRANSPORT IN SYNAPTOSOMES BY TETANUS TOXIN

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KEY WORDS: tetanus toxin; synaptosomes; uptake and liberation of ^{3}H-GABA.

Depression of central inhibition by tetanus toxin (TT) is due to presynaptic blockade of amino-acidergic synapses [1, 3, 11, 12]. It has been shown that slices of brain structures poisoned *in vivo* and nerve endings isolated from them liberate less γ-aminobutyric acid (GABA) on depolarization than in the control [10, 15]. Disturbance of GABA secretion has also been obtained through the direct action of TT on isolated nerve endings (synaptosomes) [5, 6].

In the present investigation the dependence of the action of TT on synaptosomes on the transmembrane ionic gradients was analyzed.

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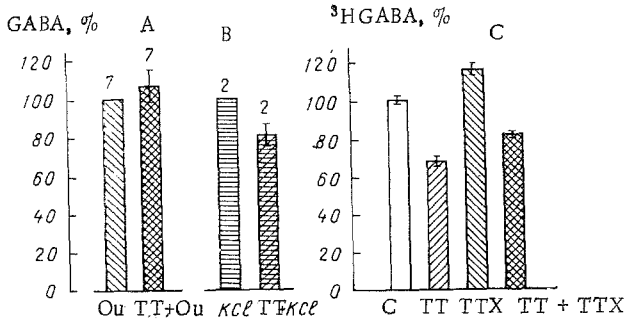

Fig. 1. Effect of TT on transport processes in synaptosomes. A) Liberation of GABA from synaptosomes induced by ouabain (Ou), B) K^+-depolarization (30 mM KCl). Liberation of GABA in presence of TT (M ± m) expressed in % of values obtained in experiments with ouabain alone or with K^+, taken as 100. Incubation time with TT 35 min. Numbers denote number of experiments; C) uptake of ^{3}H-GABA by synaptosomes preincubated with TT for 2.5 h; C) control, n = 3.

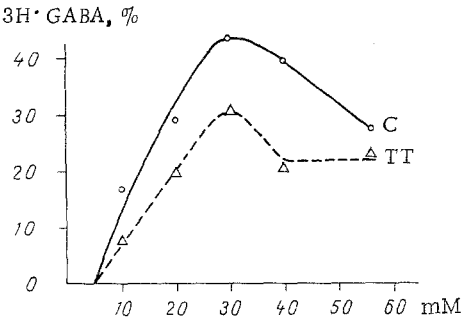

Fig. 2. Effect of TT on coupling of depolarization and secretion of ^{3}H-GABA in synaptosomes. Abscissa, increase in K^+ concentration in medium (in mM); ordinate, liberation of neruotransmitter (in % of amount of ^{3}H-GABA taken up by synaptosomes in medium with 5 mM K^+). Mean values for five experiments. Incubation time with TT 15 min.

EXPERIMENTAL METHOD

The action of purified TT [8] was studied on synaptosomes isolated from the cerebral cortex of rats [4, 13] weighing 180-200 g. In the experiments of series I synaptosomes (6 mg protein, by Lowry's method [14], in 1 ml) were incubated with TT (1200 MLD for rats in 1 ml) in 4 ml of medium of the following composition (in mM): NaCl 104, KCl 5, Na_2HPO_4 1.2, $CaCl_2$ 1.2, $MgCl_2$ 1.3, glucose 20, Tris-HCl 20, pH 7.6 (at 25°C). TT, in a dose of 100 µl in 0.1M phosphate buffer (pH 7.5), or 0.1 ml buffer alone (control) was added in the cold to glass bottles containing a suspension of synaptosomes in incubation medium (IM), and 15-60 min later incubation (35 min) began in a water bath of 37°C, with energetic mixing of the samples on a magnetic mixer. To study GABA liberation, ouabain or KCl (final concentration 0.5 and 30 mM respectively) was added to the bottles 20 min after the beginning of incubation, and incubation was then continued for a further 15 min. The synaptosomes were then separated from IM by centrifugation at 16,000 rpm (TsLR-1 centrifuge) and the GABA content in the synaptosomes and IM was determined as described previously [4].

In the experiments of series II and III the compositions of the IM were as follows (in mM): 1) NaCl 155, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1.3, NaH_2PO_4 1.2, glucose 10, Tris-Hcl 20, pH 7.6 (at 25°C); 2) the same composition, but without Ca^{++} and with 2 mM EGTA (-Ca^{++} + EGTA); 3) the same composition, but without Ca^{++} and with 2 mM EDTA (-Ca^{++} + EDTA). To depolarize the

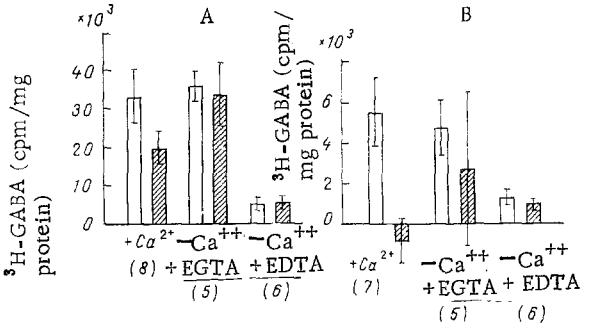

Fig. 3. Ca^{++}-dependence of action of TT on transport processes in synaptosomes. Time of preincubation of synaptomes with TT in media with different Ca^{++} concentration: ($+Ca^{++}$), ($-Ca^{++}$ + EGTA), and ($-Ca^{++}$ + EDTA) was 2.5 h. A) Uptake of ^{3}H-GABA by synaptosomes (5 min) in IM of corresponding compsitions; B) liberation of ^{3}H-GABA from synaptosomes induced by K^+-depolarization (56 mM K^+, 2 mM Ca^{++}). Numbers denote number of experiments (unshaded columns — control, shaded columns — TT).

synaptosomal membranes solutions with K^+ concentration increased to 10, 20, 30, 40, and 56 mM were used. The osmolarity of the solutions was kept constant by an equivalent decrease in the Na^+ concentration.

In the experiments of series II 0.1 ml of a suspension of synaptosomes (about 0.2 mg protein) in 0.32 M sucrose was added to glass tubes containing in 0.9 ml IM ($-Ca^{++}$ + EGTA) ^{3}H-GABA (New England Nuclear, Boston, Mass., specific activity 34.5 mCi/mmole, final concentration $1 \cdot 10^{-8}$ M) and 1000 MLD of TT in 1 ml. Incubation was carried out at 37°C for 15 min, after which the synaptosomes were separated from IM on Synpor filters (Czechoslovakia) with a pore diameter of 0.85 μ. The choice of filter is explained by the fact that its retention of radioactivity of the synaptosomes was only 10% less than on filters with a smaller pore diameter (Vladipor — 0.55μ), and the rate of filtration was much higher, which is important when transport of labeled compounds is studied. Residues of synaptosomes on the filters were washed 3 times with portions each containing 3 ml IM ($-Ca^{++}$ + EGTA); each washing lasted 10 sec. In the experiments to study uptake of ^{3}H-GABA, a 4th washing also was carried out with IM ($-Ca^{++}$ + EGTA), but with 10 ml for 20 sec. In the study of Ca^{++}-dependent liberation induced by K^+-depolarization, a 4th washing was carried out in the same way as when uptake was studied, but with solutions containing Ca^{++} and with an increased potassium concentration.

In the experiments of series III synaptosomes (1 mg protein in 1 ml) were incubated with TT (1000 MLD in 1 ml) for 2.5 h in glass bottles at 37°C in 3 ml of IM ($+Ca^{++}$), ($-Ca^{++}$ + EGTA), and ($-Ca^{++}$ + EDTA). Tetrodotoxin (TTX, final concentration 10^{-6}–10^{-7} g/liter)was added to some samples. All TT not bound with membranes of synaptosomes after 2.5 h was removed by washing: 7 ml IM of the same composition was added to the experimental and control samples, which were then centrifuged for 1-5 min at 10,000-15,000g (K-24 centrifuge, East Germany) at 0-2°C. The residues were resuspended in 1 ml of IM, a further 9 ml of IM was added, and centrifugation was carried out in the same way. The residues were then resuspended in 0.32 M sucrose and diluted so that 0.1 ml of suspension added to the sample contained 0.2 mg protein. The uptake and liberation of ^{3}H-GABA were investigated in the same way as in series II, but the duration of incubation when studying uptake was reduced to 5 min. Filters with residues of synaptosomes after washing were dried in air and placed in flasks with toluene scintillator [4]. The radioactivity of the residues of synaptosomes on the filters was measured by the SL-30 scintillation spectrometer (Intertechnique, France). The significane of differences was assessed by the criterion of signs (P_{cs}) and by Student's t test (P_t).

EXPERIMENTAL RESULTS

In the experiments of series I the effect of TT was compared on two methods of GABA liberation from synaptosomes: that due to inhibition of uptake in the presence of ouabain, and the Ca^{++}-dependent liberation caused by depolarization, an analog of the secretory process. Ouabain causes powerful liberation of GABA into IM: More than 70% of the total GABA content

in the suspension is found in IM [4]. Liberation of GABA induced by ouabain was not inhibited by TT (Fig. 1A). This result can probably be explained on the grounds that, because of the resistance of Na^+,K^+-ATPase to TT [2] its inhibition by ouabain leads to equal changes in the transmembrane gradients of Na^+ and K^+, and to closely similar shifts in the distribution of GABA between synaptosomes and medium determined by them. Conversely, liberation of GABA induced by K^+-depolarization was partially suppressed by TT (Fig. 1B).

In the experiments of series II incubation of the synaptosomes with TT for 15 min was not accompanied by significant changes in uptake of ^{3}H-GABA by the synaptosomes (a decrease of 8.6%, $P_t > 0.1$), but the values for liberation of ^{3}H-GABA induced by an increase in the external K^+ concentration were lower throughout the range of depolarization tested (Fig. 2). For K^+ concentrations of 30 and 40 mM, the difference in the quantity liberated by the control and poisoned synaptosomes was statistically significant ($P_{cs} = 0.05$), but later it decreased. It must be emphasized that the existence of a certain optimal zone of K^+ concentration to reveal the effect of TT has also been demonstrated during the study of acetylcholine secretion in the rat neuromusclar junction [7] and also for mediator liberation from shredded slice [9]. The results of this series of experiments, indicating the possibility of comparatively rapid manifestation of the action of TT on ^{3}H-GABA liberation, agree with data showing changes in membrane potenial of the synaptosomes under the influence of TT [16] and depression of inhibition in brain structures following direct application of TT to them [10].

In the experiments of series III, investigation of ^{3}H-GABA transport was preceded by incubation of synaptosomes for 2.5 h in IM differing in their CA^{++} concentration. Incubation of synaptosomes with TT in Ca^{++}-containing medium was accompanied by a significant decrease in their ability to accumulate ^{3}H-GABA (by 40%, $P_{cs} = 0.01$). These data agree with the results of other investigations [5, 6, 9]. No significant effect of TT on ^{3}H-GABA uptake could be detected in IM ($-Ca^{++}$ + EGTA) and IM ($-Ca^{++}$ + EDTA) (Fig. 3A). A sharp fall in uptake of ^{3}H-GABA by synaptosomes in IM ($-Ca^{++}$ + EDTA) can probably be explained by chelate formation with Mg^{++}, a coenzyme for transport ATPases.

GABA uptake is absolutely dependent on the Na^+ gradient, and for that reason a decrease in GABA uptake could be one of the results of increased permeability for Na^+. With this in mind, an attempt was made to abolish the difference in uptake between poisoned and control synaptosomes by blocking entry of Na^+ into synaptosomes by means of TTX, a specific blocker of Na^+ channels. Addition of TTX to the incubation medium (and during accumulation of ^{3}H-GABA) led to an increase in ^{3}H-GABA uptake by the synaptosomes (Fig. 1B), but in the presence of TTX the uptake of ^{3}H-GABA by the poisoned synaptosomes remained lower than in the control ($P_t < 0.01$). Since the tendency for uptake to decrease in the presence of TT was observed in IM ($-Ca^{++}$ + EGTA) also, it can be postulated that the primary action of TT, depolarizing the membrane, for example [17], is not Ca^{++}-dependent. In that case the reduction in uptake may be the result of a process of Ca^{++}-dependent ^{3}H-GABA liberation parallel to its uptake. The decrease in the glycine concentration in the poisoned half of the spinal cord of animals with local tetanus [17] may perhaps reflect similar liberation of mediator at rest.

Synaptosomes incubated for a long time with TT in medium containing Ca^{++} liberated less of the accumulated ^{3}H-GABA on K^+ depolarization (Fig. 3B, $P_t < 0.01$), in agreement with our own data for synaptosomes [5, 6] and with the results of investigations on shedded slices [9]. The tendency for ^{3}H-GABA liberation to be distrubed also was noted in experiments with synaptosomes incubated with TT in IM not containing Ca^{++} (Fig. 3B). It is important to note that the decrease in ^{3}H-GABA liberation was not simply the result of its reduced uptake, but rather a case of true inhibition of liberation: ^{3}H-GABA liberation from poisoned synaptosomes, expressed as a percentage of the ^{3}H-GABA accumulated by them, was 64.3% less than in the control ($P_{cs} = 0.05$, n = 8).

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SEROTONIN RECEPTORS OF LYMPHOCYTES: A RADIORECEPTOR STUDY

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KEY WORDS: lymphocytes; serotonin receptors; ^{3}H-serotonin; radioreceptor method.

According to data in the literature several receptors for neurotransmitters exist on the surface of lymphocytes. For instance, the use of the radioreceptor method has shown the presence of β-adrenoreceptors [4] and acetylcholine receptors [7] on immunocompetent cells and evidence has been obtained of the existence of opiate receptors of lymphocytes [2] and of nonopiate receptors for β-endorphin [8]. Data also have been published on the effect of adrenomimetics [4] and of methioineenkephalin [2] on the adenylate cyclase system of lymphocytes.

The existence of sites of high-affinity specific binding of ^{3}H-spiroperidol [6], a marker of brain serotonin receptors [11], on lympocytes has recently been demonstrated. However, doubts have recently been expressed on the existence of serotonin receptors on lymphocytes, for specific binding of ^{3}H-spiroperidol was neither stereochemically specific nor saturating. Binding sites of this ligand also were characterized by high values of inhibition constants of serotonin and other drugs with high affinity for brain serotonin receptors [5].

Despite the proven action of serotonin on development of the immune response [1], direct proof of the presence of serotonin receptors on lymphocytes thus has not so far been obtained.

Accordingly, in the investigation described below an attempt was made to obtain proof of the existence of serotonin receptors of human lymphocytes by radioreceptor analysis of binding of ^{3}H-serotonin (^{3}H-5HT) with these cells.

EXPERIMENTAL METHOD

Lymphocytes were isolated from heparinized peripheral blood from healthy donors by centrifugation in a Ficoll—Verografin gradient. To isolate the coarse membrane fraction of lymphocytes the cells were disintegrated by ultrasoudn [9]. The suspension of disintegrated lymphocytes was centrifuged at 700g for 20 min. The supernatant was kept and the residue resuspended in mM 50 Tris-HCl (pH 7.4) on a "Type 302 Homogenizer" (Poland) with Teflon-glass attachment, at 2000 rpm (1.5 μ), and then centrifuged again. The resulting supernatants were

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