ACTION OF NEUROTROPIC DRUGS ON K^+-INDUCED GABA RELEASE FROM BRAIN SYNAPTOSOMES

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It was shown previously that psychotropic drugs of different classes have varied effects on active GABA transport through the synaptosomal membrane [6-8]. Compounds which are the most active inhibitors of GABA uptake by synaptosomes also caused the greatest release of this mediator [8]. Neuroleptics and antidepressants, which behaved like depolarizing agents, inducing GABA release from synaptosomes in the same way as during K^+-depolarization [8], were most effective from this point of view. Some of these compounds (chloropromazine and imipramine), in effective concentrations, were shown in fact to reduce the transmembrane potential in synaptosomes [9].

Virtually all investigations of the action of psychotropic drugs on GABA transport through the nerve ending membrane have been undertaken on native synaptosomes. Nevertheless, a more appropriate target for psychotropic drugs in order to develop methods of treatment is presented by functionally or structurally changed nerve endings and, in particular, those which have been de- or hyperpolarized for a long time.

The aim of this investigation was to study the action of chloropromazine, fluacizine, trifluperidol, and imipramine on K^+-induced release of GABA from brain synaptosomes.

EXPERIMENTAL METHOD

Synaptosomes were isolated from the cerebral cortex of noninbred male albino rats weighing 190-220 g [14]. The synaptosomes obtained were suspended in incubation medium of the following composition (in mM): NaCl 100, KCl 6, $CaCl_2$ 1, glucose 10, sucrose 100, Tris-phosphate buffer, pH 7.4, 30 (20°C), at the rate of 2 ml medium per gram of cerebral cortex. [^{3}H]-GABA with specific activity of 10 Ci/mmole (New England Nuclear) was added to the suspension of synaptosomes in a dose of 2 μCi/ml. To inhibit GABA metabolism, aminohydroxyacetic acid also was added to a final concentration of 1 mM. The mixture was then incubated for 20 min at 37°C. GABA uptake was stopped by the addition of 4 volumes of ice-cold 0.32 M sucrose, after which the suspension was centrifuged for 10 min at 2000g. The residues were resuspended in 0.32 M sucrose at the rate of 2.5 ml of sucrose solution per gram of cerebral cortex.

In the experiments with psychotropic drugs 50 μl of a suspension of synaptosomes, "loaded" with [^{3}H]-GABA, was added to an incubation medium containing these compounds in the above concentration. Samples were incubated during continuous mixing for 20 min at 37°C. Release of [^{3}H]-GABA was stopped by placing the samples in a centrifuge rotor cooled to 0°C. The suspension was then centrifuged at 2000g for 10 min.

The effect of the drugs on mediator release from synaptosomes was estimated from the level of radioactivity in the supernatant. From each sample 0.2 ml of supernatant was taken and added to 10 ml of scintillation fluid containing 3 ml ethanol, 7 ml toluene, 0.5% of 2,5-diphenyloxazole (PPO), and 0.01% of 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP). Radioactivity was measured with a liquid scintillation counter (Intertechnique, France). The counting efficiency was verified by the external standard method. Results were expressed in per-

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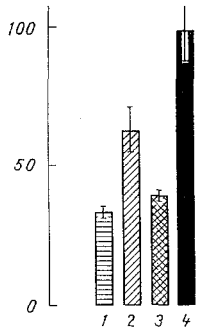

Fig. 1. Effect of conditions of incubation on [^{3}H]-GABA release from synaptosomes. 1) Spontaneous release; 2) KCl (50 mM); 3) A_{23187} (0.2 μM); 4) Triton X-100 (10% solution). Ordinate, [^{3}H]-GABA release (in %).

centages, taking as 100 either the quantity of GABA released from synaptosomes as a result of their treatment with 10% solution of the detergent Triton X-100, or the quantity of GABA released in the control (spontaneous GABA release). The radioactivity of the samples after treatment of the synaptosomes with a 10% solution of Triton X-100 and spontaneous release of GABA was 75,000 ± 7000 and 25,000 ± 2500 cpm/mg protein respectively. The protein concentration was determined by Lowry's method.

EXPERIMENTAL RESULTS

The results (Fig. 1) are evidence that treatment of synaptosomes with detergent leads to maximal release of [^{3}H]-GABA from them, three times above the level of spontaneous GABA release ($P < 0.001$). Depolarization of the synaptosomes induced by increasing the K^+ concentration in the incubation medium to 50 mM, likewise caused a twofold increase ($P < 0.001$) in [^{3}H]-GABA release from the synaptosomes (Fig. 1). These results are in good agreement with data published previously [7, 11, 12].

In the next series of experiments incubation of native synaptosomes with chloropromazine was found to lead to a twofold increase ($P < 0.01$), and with fluacizine, trifluperidol, and imipramine, to a 50% increase ($P < 0.05$) in ^{3}H-GABA release from these structures Table 1).

The experiments showed that both an increase in the K^+ concentration in the incubation medium of the synaptosomes [9, 11] and addition of a number of psychotropic drugs, especially chloropromazine and imipramine [9], caused a decrease in transmembrane potential in the synaptosomes, leading to their depolarization. In the first case this took place on account of a decrease in the K^+ gradient of the membrane, in the second case, evidently, on account of nonspecific inhibition of Na, K-ATPase through a reduction in the "flowability" of its lipid environment [9]. Depolarization of native synaptosomes is known [2] to lead to secretion (release) of mediators. In the present experiments, in fact, an increase in the K^+ concentration in the incubation medium and addition of psychotropic agents caused a significant increase in the efficiency of ^{3}H-GABA release from the synaptosomes (Fig. 1; Table 1).

The action of the drugs mentioned above, in conjunction with a K^+ concentration of 50 mM, leads to additional depolarization of the synaptosomal membrane (unpublished data obtained with the aid of potential-sensitive fluorescent probes). Hence additional release of the mediator would be expected. Instead, however, the simultaneous action of two depolarizing factors led to a marked decrease in [^{3}H]-GABA release from the synaptosomes. The cause of this effect, in all probability, has nothing to do with a change in permeability of the synaptic membrane for K^+ and Na^+, the principal potential-forming ions, for, as we found, the result of the simultaneous influence of K^+-depolarization and the psychotropic drug on transmembrane potential obeys the law of additivity. Consequently, uncoupling of excitation (depolarization) and secretion were observed. The factor coupling these processes is known to be Ca^{++} entry into the nerve ending [1] along potential-dependent Ca^{++}-channels [3].

TABLE 1. Effect of Psychotropic Drugs on [^{3}H]-GABA Release from Native Synaptosomes and K^+-Depolarized Synaptosomes (M ± m)

Experimental conditions	[^{3}H]-GABA release, %
Control (25)	100±6,2
K+ (25)	190±23,6
Chloropromazine (6)	190±17,7
Chloropromazine + K^+ (5)	118±12*
Fluacizine (5)	162±11,9
Fluacizine + K^+ (5)	78±11,9*
Trifluperidol (5)	156,6±30
Trifluperidol + K^+ (7)	104±6,9**
Trifluperidol + K^+ + A_{23187} (7)	152±5***
Imipramine (8)	135±15,8
Imipramine + K^+ (8)	112±17,1**
Imipramine + K^+ + A_{23187} (8)	152±10,6***

Legend. Fluacizine, trifluperidol, and imipramine used in a concentration of 0.5 mM, chloropromazine 0.25 mM, K^+ 50 mM, and A_{23187} 0.2 μM. Number of experiments given in parentheses. *$P < 0.05$, **$P < 0.01$ compared with K^+, ***$P < 0.01$ compared with use of K^+ in combination with drug (significance of differences determined by Wilcoxon's T test).

It was found previously that Met-enkephalin, stimulating GABA release from brain synaptosomes in concentrations of about 10^{-12}-10^{-4} M, in a concentration of 5×10^{-10} M inhibited by 50% K^+-induced GABA outflow [13]. Other workers [4] have shown that opioid peptides, accelerating Ca^{++} uptake by synaptosomes obtained from rat brain in physiological concentrations of extrasynaptosomal K^+ inhibit this process against the background of K^+-depolarization of the synaptosomes. Inhibition by Met-enkephalin of K^+-induced GABA release was thus due in all probability to blocking of conductivity of the synaptic membrane for Ca^{++}. Under the influence of the psychotropic drugs studied on K^+- depolarized synaptosomes, blocking of Ca^{++}-channels also takes place and, as result of this, the efficiency of [^{3}H]-GABA release remains at the spontaneous level.

To test the validity of this hypothesis, the Ca^{++} ionophore, antibiotic A_{23187} [10] was added to the incubation medium of the synaptosomes. As a result a small increase ($P < 0.05$) in the efficiency of [^{3}H]-GABA release from the synaptosomes was observed compared with the control (Fig. 1). This increase, however, was much less than the increase in [^{3}H]-GABA release observed after K^+-depolarization of the synaptosomes, or as a result of their treatment with the substances described above. Addition of A_{23187} to the incubation medium along with K^+ and with trifluperidol, or K^+ and imipramine, prevented the uncoupling of depolarization and secretion and caused a marked increase in the efficiency of [^{3}H]-GABA release, which reached the level characteristic of the action of psychtropic drugs on native synaptosomes (Table 1).

The results are thus evidence that the absence of an effect of the psychotropic drugs on [^{3}H]-GABA release from synaptosomes in the presence of K^+-depolarization is evidently due to blocking of conduction of the synaptic membrane for Ca^{++}. The mechanism of this phenomenon is not yet clear.

The results also suggest that both neuroleptics and antidepressants can affect resting and pathologically excited nerve endings in various ways, uncoupling electrical and chemical transmission of the nervous impulse in the "excited" synapse and inducing mediator secretion from "resting" nerve endings.

This state of affairs demonstrates the need to investigate the effect of pharmacological agents on the correspondingly modified synaptic structures, for the mechanism of their action on pathologically modified targets may differ in principle from their mechanism of action on native targets. In particular, one promising approach would seem to be to study the mechanism of action of anticonvulsants on K^+-depolarized synaptosomes or on synaptosomes isolated from foci of hyperactivity: generators of pathologically enhanced excitation [5].

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ANXIOLYTIC ACTIVITY OF SOME β-CARBOLINES IN RATS

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β-Carbolines are condensation products of indoles (in particular, serotonin) with aldehydes, they are found in plant and animal tissues [5], and they are of great importance for the regulation of certain processes of higher nervous activity, including the formation of alcohol motivation [3, 4]. The role of endogenous ligand of benzodiazepine receptors (BD-receptors) has been ascribed to certain natural β-carbolines [10, 11]. Meanwhile selective high-affinity binding of certain synthetic analogs of β-carbolines with BD-receptors has been described in certain regions of the brain, but the relationship between ligand and anxiolytic properties has not yet been adequately studied. The aim of the present investigation was accordingly to study this problem.

EXPERIMENTAL METHOD

Anxiolytic activity was studied by a method which is a variant of the conflicting situation: the appearance of motivated fighting between rats of a pair for safe territory (a bench above an electrode floor) during electronociceptive stimulation of the limbs. If both rats of the pair jump together onto the safe bench in the course of 1 min and remain on it for 10 sec, this is assessed as the manifestation of an anxiolytic effect [2]. Antineurotic action was studied as the ability of the drugs to influence an avoidance reaction formed in one rat during nociceptive stimulation of the other [1]. During investigation of the antineurotic effect of the compounds their doses were 5-8 times higher than ED_{50} values characteristic of the anxiolytic effect of the drugs [4].

Experiments were carried out on noninbred male albino rats weighing 180-200 g (antineurotic effect, effect of bicuculline) or weighing 480-500 g (anxiolytic action on a model of

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