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The Influence of Antidepressive Treatment on GABA-Related Mechanisms in the Rat Hippocampus: Behavioral Studies

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PŁAŹNIK, A., W. KOSTOWSKI AND R. STEFAŃSKI. *The influence of antidepressive treatment on GABA-related mechanisms in the rat hippocampus: Behavioral studies.* PHARMACOL BIOCHEM BEHAV **33**(4) 749–753, 1989.—The effect of antidepressive treatment upon central GABAergic mechanisms has been studied in a behavioral model. Local injections (dentate gyrus of the dorsal hippocampus) of picrotoxin, a chloride channel blocker linked with the function of GABA-A receptor complex, potently stimulated rat motility recorded in the automated open fields. The intra-hippocampal administration of GABA antagonized the behavioral effect of picrotoxin (0.5 μg). Similar effects were produced by addition to picrotoxin solution (0.25 μg) of the GABA-A receptor agonist muscimol (0.25 μg). Chronic (21-day), but not single, treatment of rats with desipramine (10 mg/kg, IP, daily) significantly attenuated picrotoxin-induced locomotor stimulation, when the GABA antagonist was given 24 hr after the last dose of the antidepressant. Repeated electroconvulsive shocks did not significantly change picrotoxin effect, while single shock produced some degree of inhibition of drug-induced motor stimulation. It is hypothesized that chronically applied desipramine, but not electroconvulsive shocks, may enhance the activity of hippocampal GABA-A receptor-related system.

Hippocampus Picrotoxin Desipramine ECS Locomotor activity Rat

THE facilitatory involvement of brain GABA-ionophore complex in the central effects of antidepressant treatment has been repeatedly proposed by many authors (3, 11, 15, 21, 25). The most often cited facts in favor of this concept have been recently summarized and discussed by Bartholini *et al.* (1) and Lloyd *et al.* (11). These are among others: (1) up-regulation of GABA-B receptor sites and decreased density of benzodiazepine receptors after chronic antidepressants, (2) therapeutic effects in depression of GABA receptor agonists, e.g., fengabine, progabid, (3) the "antidepressive"-like influence of GABAergic compounds in animal models of depression, (4) and reported by some authors, decreased cerebrospinal fluid levels of GABA in depressed patients [cf. (1)]. However, recently some of these facts, including antidepressant-induced changes in the GABA-B and benzodiazepine receptors, have not been reproduced in other laboratories (4, 5, 18). Moreover, it has been proposed that contrary to the aforementioned hypothesis, blockade but not facilitation of excessive GABAergic inhibition of reward systems may contribute to clinical effects of antidepressants (24). Accordingly, it was shown that 23 clinically effective antidepressants blocked GABA-related changes in the function of receptor-ionophore complex (antagonism of the inhibitory action of GABA on ^{35}S-t-butylbicyclophosphorothionate (TBPS) binding (24). Thus, the antidepressants produced similar changes to that of well-known GABA antagonists. The fact that almost all antidepressants can induce convulsions, or are proconvulsants, supports the possibility of the anti-GABA activity of these compounds.

In the light of these disparate findings we have decided to study the relationship between GABA-related system and antidepressants in a functional model. The effects of acute and chronic treatment of rats with desipramine and electroconvulsive shocks (ECS) upon locomotor stimulation produced by intra-hippocampally administered picrotoxin were compared. Intra-hippocampal injections of picrotoxin are known to produce short-latency increase in rat locomotion, followed after higher doses (above 1 μg/side) by convulsions (6,7). The mechanism of this phenomenon involves an enhancement of disinhibitory processes in the structure, stimulus propagation, due to antagonism of GABA-A ionophore complex-related postsynaptic inhibition (6,27). According to the Polc model [see (20)], the complex consists of GABA-A receptors, benzodiazepine, picrotoxin, barbiturate and chloride ion recognition sites located on neuronal membranes. On the cellular level, picrotoxin blocks the function of chloride channel, the output system of GABA-A receptor-related complex (2, 23, 26). Thus, we have studied the affect of the well-recognized antidepressant treatment upon the function of GABA-A-related ionophore, in the place where all the intrinsic and ionic mechanisms converge. This may be considered as a preliminary step for the more detailed analysis of the contribution of the elements of receptor complex to the central effects of antidepressant drugs and

ECS. The selection of this behavioral reaction, linked to the GABA-A receptor system, was based on several premises. Recently, chronic treatment of rats with desipramine was found to reduce THIP- (GABA-A receptor agonist) induced antinociception (3): Furthermore, the control of brain electrophysiological processes is believed to depend mostly on the integrity of GABA-A receptor complex, while central functions of GABA-B receptors are much less recognized. To ascertain the specificity of the effect of picrotoxin, an attempt was made to block drug-induced hyperlocomotion by local administration of GABA and muscimol (a selective GABA-A receptor agonist) prior to or together with picrotoxin injection.

METHOD

Animals

Male Wistar rats weighing 200–220 g were used for the experiment. Following socket implantation the rats were kept individually in wire-mesh cages to avoid damage to the socket. The animals were kept in standard laboratory conditions with water and food ad lib. All experiments were performed between 0900 and 1300 hour.

Preparation of Animals

The animals were implanted as described previously (16). Briefly, the rats were operated upon under ethyl ether anesthesia and implanted with a socket with two metal guide cannulas (0.4 mm internal dia.) 1.5 mm above the dentate gyrus of the dorsal hippocampus (A 3.5 mm; L 2.8 mm; V 2. mm below the skull) (14). The rats were subjected to testing 7 days later.

Microinjection Procedure

Microinjections were given bilaterally with two Hamilton microsyringes connected via polyethylene tubings to two metal injections needles (0.3 mm external dia.) inserted 1.5 mm below the tip of the guide cannulas. All drugs for microinjections were dissolved in saline immediately before their administration and control rats were treated with saline only. Each rat was injected twice, with an interval of at least 2–3 weeks prior to subsequent treatment. Solutions (0.5 μl) were administered bilaterally over 30 sec. The injection needle remained in place for an additional 30 sec before it was removed and the stylet replaced. Behavioral tests were started 5 min after an intracerebral injection.

Drugs and Electroconvulsive Shocks

Picrotoxin (PX) (Serva), muscimol hydrobromide (Serva), and GABA (Fluka) were freshly prepared in saline. GABA (15 μg) was microinjected 5 min before PX (0.5 μg) and 8–10 min prior to test. In the part of the experiment with picrotoxin and muscimol, both drugs were applied simultaneously in a dose of 0.25 μg each, in one solution. Appropriate control groups were examined parallely.

Desipramine hydrochloride (DI, Ciba-Geigy) was administered IP in a dose of 10 mg/kg daily and dissolved in saline (2 ml/kg) for 21 days. Twenty-four hr after the first (acute treatment) and 21st (chronic treatment) dose, the animals were microinjected with PX (0.5 μg) and subjected to behavioral testing.

Electroconvulsive shocks (ECS) (0.3 sec, 150 mA, 50 Hz) were administered to rats every second day through ear-clip electrodes. Intracerebral drug injections (PX, 0.5 μg) and behavioral studies were done 24 hr after the first (acute treatment) and the fifth (repeated treatment) electroshock. The ECS produced clonic-tonic fits of seizures in all rats.

Open-Field Test (OFT)

The open-field test was performed for 15 min in an automatic box (80 cm diameter round arena with 30 cm high walls) equipped with three horizontally placed photocells, in a sound-proof chamber under dim light and white noise conditions, at the same time of a day. The number of photocell interruptions was recorded and used as an index of locomotor activity. The animals were also observed from the adjacent room through a TV system and their behavior was recorded on video. The animals have not been previously habituated to the experimental conditions.

Histological Analysis

All animals were killed after the final testing day, their brains were removed and stored in 5% formalin solution, and then checked histologically. The frozen tissue was dissected into slices and the place of injection inspected with Meoflex (×40). This apparatus is comprised of a magnifying glass and slide projector.

Statistical Analysis

All data were analysed by the one-way ANOVA followed by the Duncan test. All data are expressed as mean ± SEM.

RESULTS

Histological analysis showed that the site of injection and extent of tissue damage was essentially the same as observed in our previous experiments (16). About 10% of rats was rejected due to incorrect site of injection.

All applied doses of picrotoxin (0.5, 1 and 2 μg) stimulated rat activity in the OFT (Fig. 1). The stimulation consisted of bouts of forward-directed locomotion, without orienting reactions, interrupted by periods of immobility (data not shown). After the highest dose (2 μg) the rats exhibited seizure-like activity, including "wet dog" shakes, reciprocal forepaw abduction, forced head and body turns, and circling. Major convulsions did not appear. This behavior continued after the open-field test. The rats given 2 μg of picrotoxin did not also show tendency to decrease locomotor hyperactivity during 15-min testing. Local pretreatment of rats with GABA (15 μg) antagonized the behavioral effect of 0.5 μg of picrotoxin (Fig. 2). There was also some tendency for GABA alone-treated rats to be less active than control group, however, the difference did not reach the significance level except for the first 5 min of the test. Likewise, the addition of 0.25 μg of muscimol to solution containing 0.25 μg of picrotoxin inhibited locomotor stimulation produced by nonselective GABA antagonist (Fig. 2). Muscimol also inhibited rat motor activity by itself.

Chronic, but not acute administration of desipramine significantly attenuated the stimulatory effect on locomotion of intrahippocampally injected picrotoxin (Fig. 3). The drug did not affect rat behavior on its own. In contrast to these findings, repeated ECS did not consistently change the picrotoxin effect, and the ECS + PX-treated group appeared to be the most active in the OFT (Fig. 4, lower part). On the other hand, single ECS attenuated, to some extent, the behavioral action of picrotoxin (Fig. 4, upper part).

DISCUSSION

Intra-hippocampal injections of picrotoxin potently stimulated rat locomotion. The present data corroborate other authors' findings showing that bilateral infusion of picrotoxin, in a dose of 0.1 and 1.0 μg to the dentate gyrus, strongly enhanced rat locomotor

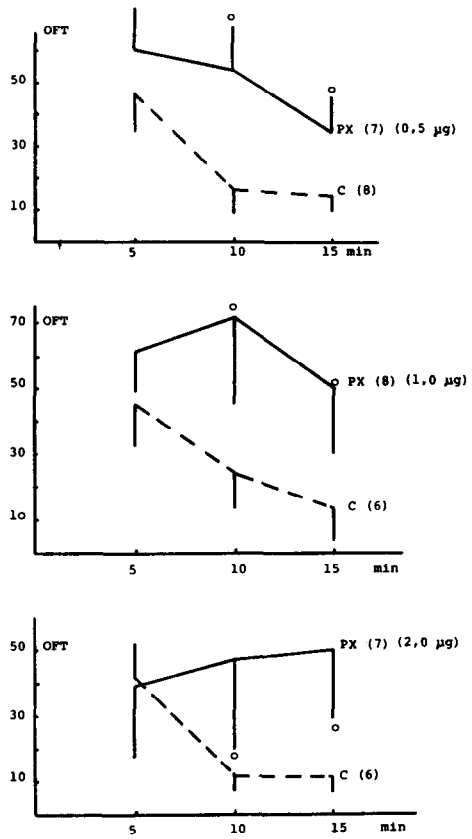

FIG. 1. The effect of intra-hippocampal injections of picrotoxin upon rat locomotion in the open-field test. Ordinate—number of photo beam interruptions; abscissa—time in min; C—control; PX—picrotoxin; number of rats is shown in the brackets; ○—differs from control. ○ = $p<0.05$.

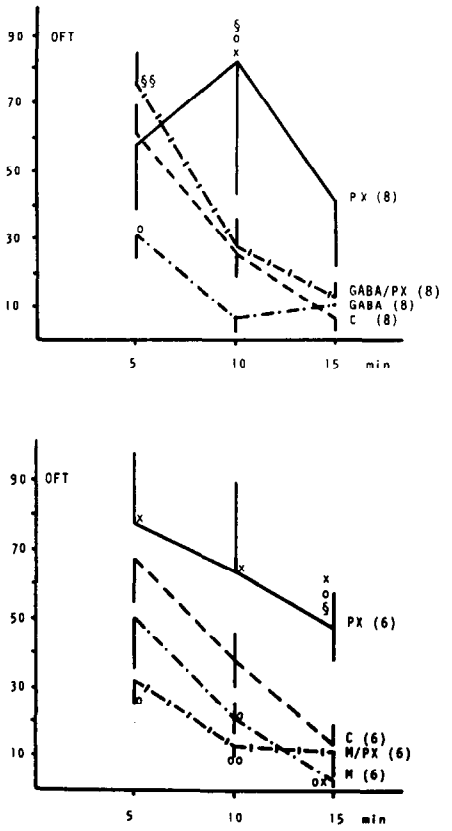

FIG. 2. The effect of local pretreatment of rats with GABA and muscimol upon picrotoxin-induced hyperlocomotion. GABA in a dose of 15 μg was given 5 min prior to picrotoxin (0.5 μg) and 8–10 min before test. Muscimol (M, 0.25 μg) was added to the solution containing 0.25 μg of picrotoxin. x—differs from GABA/PX or M/PX; §—differs from GABA or M. ○, x, § = $p<0.05$, §§ = $p<0.01$. All other explanations as in Fig. 1.

activity; the effect being most pronounced during the first 10 min (6,7). Moreover, in these experiments, similarly to our findings, no clear-cut dose-response effect was present. Locomotor hyperactivity is consistently observed after lesion to the hippocampus and it is believed to be part of a syndrome of impaired response inhibition due to a failure to habituate to novel environmental stimuli (8,9). The effect of picrotoxin, a well known GABA-ionophore antagonist, depolarizing neuronal membranes (see Introduction), may be viewed, therefore, as secondary to the drug-induced functional lesion of the limbic nucleus. It is noteworthy that the hippocampus is believed to be a key structure for modulation, transforming and transmitting of sensory output from associative cortical areas via nucleus accumbens and ventral pallidum, down-stream to brainstem centers regulating animals' motor activity (13). This, however, does not seem to be the case, since the bilateral infusion into the dentate gyrus of 1% solution of the local anesthetic lidocaine, instead of stimulation, produced significant decrease in locomotor activity during the 40-min experimental session (6). The direct relationship of picrotoxin effect with GABAergic mechanisms is shown by attenuation of drug-induced hyperlocomotion by preceding microinjection of GABA. Thus, the effect of a relatively high dose of the nonselective GABA antagonist appeared to be susceptible to manipulation with GABAergic activity. The part of the experiment with muscimol adds more arguments for such interpretation of the present data. The GABA-A receptor agonist, apart from blockade of picrotoxin effect, significantly depressed rat locomotion by itself; this effect being contrary to the influence of the nonselective GABA antagonist. Moreover, the inhibitory action of muscimol on picrotoxin stimulation was present as quick as 5 min after injection, i.e., at time when the drug did not affect animal activity on its own. From all these data taken together, it may be concluded that GABA-A receptor complex is most likely to be involved in the effect of picrotoxin and in some of the phenomenons discussed below as well.

Chronic, but not acute treatment of rats with desipramine significantly antagonized picrotoxin-induced locomotor stimulation. The mechanism of this interaction may involve an enhancement of local GABA release by desipramine (10). Consequently, it has been found that 48-hr exposition of cultured neurons from embryonic rat brain to GABA or muscimol down-regulates the benzodiazepine-GABA-chloride channel receptor complex, as shown by a decrease in the specific binding of ^{3}H-muscimol and ^{35}S-TBPS to the GABA-A and chloride channel sites, respectively (12). It is noteworthy that both ^{35}S-TBPS and picrotoxin bind to a site which is allosterically linked to GABA-A and chloride recognition sites in receptor complex located on neuronal membranes (2, 22, 26). It was also found that chronically, but not acutely given desipramine reduced THIP- (GABA-A receptor agonist) induced antinociception (3). Furthermore, the combination of muscimol and tricyclics administered at subeffective doses resulted in an additive interaction between these two classes of

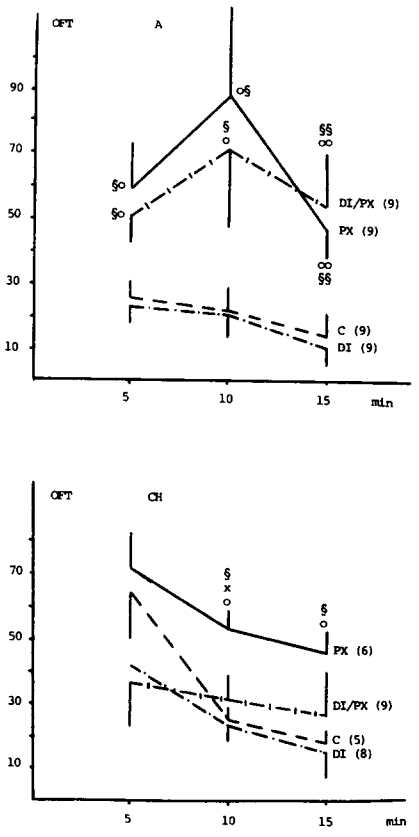

FIG. 3. The effect of acute (A) and chronic (CH) treatment of rats with desipramine (DI) upon picrotoxin-induced hyperlocomotion (PX, 0.5 μg). §—differs from DI; x—differs from DI/PX. ○, x, §=$p<0.05$; ○○, §§=$p<0.01$. All other explanations as in Fig. 1.

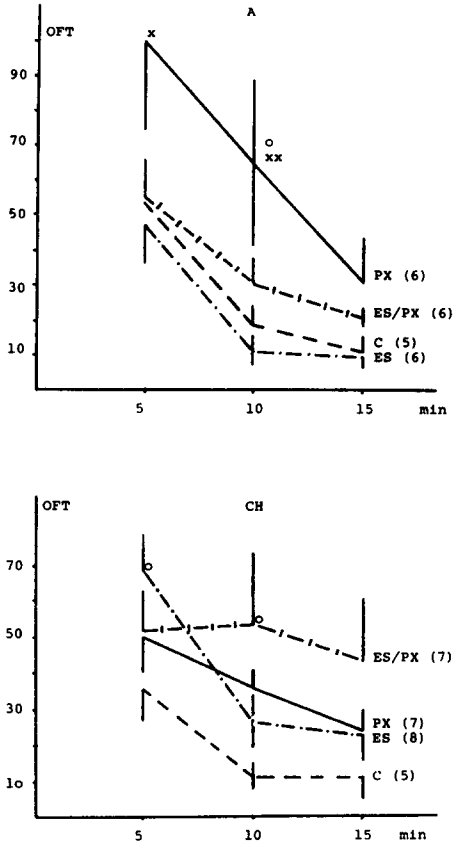

FIG. 4. The effect of single (A) and repeated (CH) treatment of rats with electroconvulsive shocks (ECS) upon picrotoxin-induced hyperlocomotion (PX, 0.25 μg). x—differs from ES. x, ○=$p<0.05$; xx=$p<0.01$. All other explanations as in Fig. 1.

drugs in a learned helplessness model of depression (19).

In contrast to the effect of desipramine, repeated treatment of rats with ECS did not univocally affect the stimulatory influence on locomotion of locally administered picrotoxin. In fact, ECS produced rather inconsistent results, except for a transient attenuation of picrotoxin-induced stimulation after single shock. The group of picrotoxin + chronic ECS-treated rats appeared also to be the most active throughout the whole experiment. One might suppose that the treatment with ECS was too short to produce any significant effect. However, it does not seem to be the case, since similar procedure of ECS application caused strong and significant changes in the activity of other brain neurotransmitter systems [e.g., dopaminergic system (17)].

Taken together, the discussed data support the role of hippocampal GABA-A receptor-linked mechanisms in the central effects of desipramine but not electroconvulsive shocks. It seems that the lack of uniform changes in the functional model after desipramine and ECS may be tentatively interpreted as opposing the aforementioned suggestions about the direct involvement of this brain neurotransmitter in the behavioral effects of all kinds of antidepressant treatments. However, the fact that such a corollary can be based on the results obtained with desipramine and ECS only does not allow us to draw more firm conclusion, even though both desipramine and ECS are believed to belong to the most potent antidepressive therapies. Moreover, it is quite possible that central mechanisms of antidepressant treatment are more closely related to the function of GABA-B receptor system, as it has been already demonstrated in receptor binding studies (see Introduction). Apparently, more research is needed to solve this important problem.

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REFERENCES

1. Bartholini, G.; Lloyd, K. G.; Scatton, B.; Zivkovic, P.; Morselli, P. L. The GABA hypothesis of depression and antidepressant drug action. Psychopharmacol. Bull. 21:385–388; 1985.
2. Borman, J. Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes. Trends Neurol. Sci. 11:112–116; 1988.
3. Borsini, F. Functional evidences for altered gabaergic receptors following chronic desipramine treatment in rats. J. Pharm. Pharmacol. 38:934–935; 1986.
4. Cross, J. A.; Horton, R. W. Are increases in $GABA_B$ receptors consistent findings following chronic antidepressant administration. Eur. J. Pharmacol. 141:159–162; 1987.
5. Cross, J. A.; Horton, R. W. Effects of chronic oral administration of

the antidepressants, desmethylimipramine and zimelidine on rat cortical $GABA_B$ binding sites: a comparison with 5-HT_2 binding sites changes. Br. J. Pharmacol. 93:331–336; 1988.
6. Flicker, Ch.; Geyer, M. A. Behavior during hippocampal microinfusions. III Lidocaine versus picrotoxin. Brain Res. Rev. 4:129–136; 1982.
7. Flicker, Ch.; Geyer, M. A. Behavior during hippocampal microinfusions. IV Transmitter interactions. Brain Res. Rev. 4:137–147; 1982.
8. Gray, J. A. McNaughton, N. Comparison between the behavioral effects of septal and hippocampal lesions: A review. Neurosci. Biobehav. Rev. 7:119–188; 1983.
9. Kimble, D. P. Hippocampus and interval inhibition. Psychol. Bull. 70:285–295; 1968.
10. Korf, J.; Venema, K. Desmethylimipramine enhances the release of endogenous GABA and other neurotransmitter amino acids from the rat thalamus. J. Neurochem. 40:946–950; 1983.
11. Lloyd, K. G.; Thuret, F.; Pilc, A. Upregulation of γ-aminobutyric acid (GABA)B binding sites in rat frontal cortex: a common action of repeated administration of different classes of antidepressants and electroshocks. J. Pharmacol. Exp. Ther. 235:191–199; 1985.
12. Maloteaux, J-M.; Octave, J-N.; Gossum, A.; Laterre, Ch.; Trouet, A. GABA induces down-regulation of the benzodiazepine-GABA receptor complex in the rat cultured neurons. Eur. J. Pharmacol. 144: 173–183; 1987.
13. Mogenson, G. J.; Nielsen, M. A study of the contribution of hippocampal-accumbens-subpallidal projections to locomotor activity. Behav. Neural Biol. 42:38–51; 1984.
14. Pellegrino, A. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1967.
15. Petty, F.; Sherman, A. D. GABAergic modulation of learned helplessness. Pharmacol. Biochem. Behav. 15:567–570; 1981.
16. Płaźnik, A.; Danysz, W.; Kostowski, W. Some behavioral effects of microinjections of noradrenaline and serotonin into the hippocampus of the rat. Physiol. Behav. 31:625–631; 1983.
17. Płaźnik, A.; Kostowski, W. The effects of antidepressants and electroconvulsive shocks on the functioning of the mesolimbic dopaminergic system: a behavioral study. Eur. J. Pharmacol. 135:389–396; 1987.
18. Przegaliński, E.; Rokosz-Pelc, A.; Baran, L.; Vetulani, J. Repeated treatment with antidepressant drugs does not affect the benzodiazepine receptors in preincubated membrane preparations from mouse and rat brain. Pharmacol. Biochem. Behav. 26:35–36; 1986.
19. Poncelet, M.; Martin, P.; Danti, S.; Simon, P.; Soubrie, P. Noradrenergic rather than GABAergic processes as the common mediation of the antidepressant profile of GABA agonists and imipramine-like drugs in animals. Pharmacol. Biochem. Behav. 28:321–326; 1987.
20. Richards, J. G.; Möhler, H. Benzodiazepine receptors. Neuropharmacology 23:233–242; 1984.
21. Sackheim, H. A.; Decina, P.; Prohovnik, I.; Malitz, S.; Resor, S. R. Anticonvulsant and antidepressant properties of electroconvulsive therapy: a proposed mechanism of action. Biol. Psychiatry 18: 1301–1310; 1983.
22. Squires, R. F.; Casida, J. E.; Richardson, M.; Saedrup, E. [^{35}S]*t*-Butylbicyclophosphorothionate binds with high affinity to brain-specific sites coupled to γ-aminobutyric acid-A and ion recognition sites. Mol. Pharmacol. 23:326–336; 1983.
23. Squires, R. F. Benzodiazepine receptors. In: Lajtha, A., ed. Handbook of neurochemistry, vol. 6: Receptors in the nervous system. New York: Plenum Press; 1984:261–306.
24. Squires, R. F.; Saedrup, E. Antidepressants and metabolites that block $GABA_A$ receptors coupled to ^{35}S-t-butylbicyclophosphorothionate binding sites in rat brain. Brain Res. 441:15–22; 1988.
25. Suranyi-Cadotte, B. E.; Dam, T. V.; Quirion, R. Antidepressant-anxiolytic interaction: decreased density of benzodiazepine receptors in rat brain following chronic administration of antidepressants. Eur. J. Pharmacol. 106:673–675; 1984.
26. Ticku, M. K.; Madsay, G. Convulsant/depressant site of action at the allosteric benzodiazepine-GABA receptor-ionophore complex. Life Sci. 33:2363–2375; 1983.
27. Wigström, H.; Gustafsson, B. Facilitation of hippocampal long-lasting potentiation by GABA antagonists. Acta Physiol. Scand. 125:159–172; 1985.