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Central- and Peripheral-Type Benzodiazepine Receptors: Similar Regulation by Stress and GABA Receptor Agonists

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RÄGO, L., R.-A. KIIVET, J. HARRO AND M. PÕLD. *Central- and peripheral-type benzodiazepine receptors: Similar regulation by stress and GABA receptor agonists.* PHARMACOL BIOCHEM BEHAV **32**(4) 879–883, 1989.—Central- and peripheral-type benzodiazepine (BD) receptors were labelled either by ^{3}H-flunitrazepam or ^{3}H-Ro 5-4864 in vitro after stress and in vivo administration of $GABA_A$ and $GABA_B$ agonists. A significant increase in the density of cerebral cortex and kidney BD binding sites was observed in rats after forced swimming stress. Similar changes in both type of BD receptors were also followed when naive (stressed) and handling-habituated (unstressed) rats were used. Stress in both models was unable to change the affinity of BD receptors in cerebral cortex, but significantly lowered it in kidneys. Acute treatment of rats with muscimol (1.5 mg/kg) or (−)baclofen (5 mg/kg) resulted in marked increase in the affinity of BD binding not only in cerebral cortex but also in kidneys. After (−)baclofen treatment the number of BD binding sites was lowered in the structures studied. In a separate study mice selected according to their behavioral response to (−)baclofen (1 mg/kg) were studied. Two weeks after the selection it appeared that baclofen responders were behaviorally more "anxious" than baclofen nonresponders. The number of BD binding sites was reduced in cerebral cortex, cerebellum, heart and kidneys in baclofen responders as compared to baclofen nonresponders. In several cases the changes in peripheral BD binding sites were even more pronounced than those in central ones. The data presented here evidence that peripheral- and central-type BD receptors are regulated similarly by GABA and some models of stress. The physiological mechanisms involved in similar regulation of central- and peripheral-type BD receptors are yet unknown.

Central and peripheral benzodiazepine receptors modulation GABA agonists Stress

SPECIFIC high affinity binding sites for benzodiazepines exist in both CNS and in a variety of peripheral organs like adrenals, kidney, heart and lungs (9). In contrast to the early years of benzodiazepine (BD) receptors research highly specific ligands are now available for both central- and peripheral-type BD binding sites. It has become clear that the pharmacology of these two types of BD binding sites is quite distinct. Clinically potent benzodiazepines, like clonazepam, are believed to exert their action through central GABA receptor linked BD binding sites (5). In contrast, Ro 5-4864, a benzodiazepine without anxiolytic effect, is found to be about 10,000 times more potent in peripheral BD binding sites (1,9). Furthermore, contrary to central-type BD binding sites in the CNS, peripheral-type BD binding sites are not modulated by GABA or chloride ions in vitro (12,14). Although some attempts have been made to determine the physiological importance and the role of peripheral BD binding sites, most efforts have been focused on central BD receptors, while the peripheral BD binding sites have until recently been almost a neglected area of research (1).

In contrast to the earlier in vitro studies, we have recently found that when BD binding sites were labelled in vivo, a pretreatment of mice with a $GABA_A$ receptor agonist muscimol is capable to modulate peripheral BD binding sites similarly to central ones (18). Several lines of evidence demonstrate that central BD and GABA receptors are affected by stress (2, 10, 13, 20). The aim of the present study was to examine the action of stress and GABA receptor agonists on central- and peripheral-type BD binding sites in rats. As the result of this study we present evidence indicating that central and peripheral BD binding sites are regulated in a similar manner by several kinds of stressful stimuli and both $GABA_A$ and $GABA_B$ receptor agonists.

METHOD

Animals and Drugs

Male albino rats weighing 220–250 g and male albino laboratory mice of 22–30 g of body weight (both from Rappolovo Farm, Leningrad) were used. The animals were maintained on food and

water ad lib at 20 ± 1°C on a reversed lighting cycle with lights off from 0800–2000 hr. Muscimol (Research Biochemicals Inc., Wayland, MA) and (−)baclofen (Ciba-Geigy, Basel, Switzerland) were dissolved in saline and injected in a volume of 10 ml per kg IP 60 and 45 min prior to sacrifice respectively.

Forced Swimming Stress

Forced swimming stress was carried out as described previously (13) with minor modifications. Briefly, the stress was produced by forcing the rats to swim in a water basin (50 × 40 × 25 cm) at 20 ± 1°C for 5 min. In contrast to the method mentioned above where rats showed clear symptoms of exhaustion, in our experiments shorter time (5 min) almost enabled us to avoid this problem. Animals were sacrificed by decapitation immediately after termination of forced swimming.

Naive and Handling-Habituated Rats

Animals for this study were prepared as described previously by Biggio *et al.* (2). The animals were divided into two groups: naive and handling-habituated rats. Handling-habituated rats were the rats habituated to all manipulations that precede killing. They were picked up from their cages, brought to the laboratory, held on a platform of a guillotine with their head forced through the hole left open by the blades and returned to their cages in animal house. This procedure was repeated twice a day during 12 days. At the end of this period the animals did not show any signs of resistance to the experimenter. It has been suggested that handling prior to the decapitation represents a stressful stimulus for naive animals (2). Therefore, naive animals may be termed as stressed and handling-habituated animals as unstressed animals.

Animal Selection Procedure

Recently we have described that it is possible to select mice according to their behavioral response to a moderate sedative dose of baclofen (19). In this experiment the mice were selected as described previously [for details see (19)]. In brief, mice were treated with (−)baclofen (1 mg/kg, IP) and tested in a multicage (1 animal per cage) photocell motor activity meter during 20 min. The data of every animal were registered individually. Animals were selected as follows: baclofen responders (0–50 counts), moderate responders (51–180 counts) and nonresponders (more than 180 counts). After the selection all the animals were housed back to their home cages. During the whole experimental period the animal population in the cages was carefully kept unchanged. It is important to mention that when five days after the experiment the selected animals were treated with saline and motor activity was tested anew, no significant differences were observed between the previously baclofen-selected animals. All the further experiments with baclofen-selected animals described here were carried out at least two weeks after the selection.

In the previous studies it was demonstrated that baclofen responders were also more sensitive to the action of diazepam and had significantly lower density of BD receptors in the forebrain than baclofen nonresponders (19). The aim of using baclofen-selected animals was to find out which subpopulation of these animals is behaviorally more "anxious" and to characterize comparatively their central and peripheral BD binding sites.

Behavioral Experiments

To characterize baclofen-selected animals behaviorally, two different methods of anxiolytic/anxiogenic activity were used. The first was carried out in an elevated plus-maze recently described by Pellow and File (15) with slight modifications. Shortly, the rats were placed on an open wooden surface for 5 min and then placed in the centre of the plus-maze. During a 4-min test period the following parameters were registered: the number of entries into and time spent in (a) open and (b) enclosed arms. The second method used was similar to that described previously by Blumstein and Crawley (3). In brief, the animal cage was partitioned into a small darkened (12.5 × 12.5 cm) compartment and a large highly illuminated compartment (35 × 35 cm). The number of transitions between the two compartments was counted during 6 min.

Benzodiazepine Receptor Binding Assay

The animals were killed by decapitation, brains and peripheral organs were rapidly removed and brain structures dissected on ice. Pooled tissue material was homogenized in ice-cold Tris-HCl buffer (pH 7.3) using a Potter-S glass teflon homogenizer to prepare tissue homogenate (4 mg wet weight per ml) for binding studies. When washed membranes were used a more concentrated (100 mg wet weight per 1 ml) homogenate was prepared as previously described and the membranes were washed subsequently 4 times by centrifugation (30,000 × g during 20 min) and resuspending the pellet in Tris-HCl (pH 7.3) buffer. Finally, the pellet was rehomogenized and diluted to the approximate protein concentration of 0.15 mg per probe. The binding assay with central BD receptors was carried out in the presence of 0.125–8 nM of ^{3}H-flunitrazepam (spec.act. 80 Ci/mmol; Amersham Radiochemicals) using a total incubation volume of 500 μl. Ten μM of diazepam was used to determine nonspecific binding. For peripheral BD receptors both ^{3}H-flunitrazepam (0.5–32 nM) and ^{3}H-Ro 5-4864 (spec.act. 81 Ci/mmol; New England Nuclear; 0.5–16 nM) were used. Nonspecific binding was determined in the presence of Ro 5-4864 (10 μM) or diazepam (10 μM) respectively. The incubation volume used was 500 μl. After 60 min incubation on ice all the reactions were stopped by rapid filtration over Whatman GF/B filters. The filters were washed with 4 × 3 ml of ice-cold Tris-HCl buffer. Specific binding was calculated by subtracting the nonspecific from total binding at each given radioactivity concentration. Protein content was measured by the Lowry *et al.* (11) method.

Statistics

Results are expressed as mean ± S.E.M. Data were statistically analysed using Student's *t*-test (two-tailed). For behavioral studies Dunnett's *t*-test was used.

RESULTS

Forced Swimming Stress

The main changes in central and peripheral BD binding sites after forced swimming are shown in Table 1. Swimming stress produced a significant increase in the number of ^{3}H-flunitrazepam binding sites not only in cerebral cortex but also in kidneys. The affinity for the ligand was lowered by swimming in both structures studied but only in kidneys the difference reached statistical significance. Similar changes in peripheral BD binding sites in kidneys were also registered when ^{3}H-Ro 5-4864 as ligand was used (data not shown).

Naive and Handling-Habituated Rats

The number of ^{3}H-flunitrazepam binding sites was elevated in naive (stressed) rats as compared to that of handling-habituated (unstressed) rats. The difference was significant in cerebral cortex

TABLE 1

THE EFFECT OF FORCED SWIMMING (AT 20°C FOR 5 MIN) ON ^{3}H-FLUNITRAZEPAM BINDING IN RAT CEREBRAL CORTEX AND KIDNEYS

Experimental Group	^{3}H-Flunitrazepam Binding B_{max} (fmol/mg)	%	K_D (nM)	%
	Cerebral Cortex			
Control	942 ± 62	100	1.83 ± 0.21	100
Swimming	1218 ± 88*	129	2.08 ± 0.14	114
	Kidneys			
Control	4606 ± 210	100	15.2 ± 0.8	100
Swimming	5695 ± 182*	142	20.7 ± 1.6*	136

In this study tissue membranes were washed 4 times with Tris-HCl (pH 7.3) buffer. Each value is the mean ± S.E.M. of at least 5 separate experiments. K_D and B_{max} values were determined by Scatchard plot analysis. *$p<0.05$ as compared to control animals.

but not in kidneys (Table 2). In naive rats the affinity of BD binding sites decreased in cerebral cortex as well as in kidneys but only in kidneys the difference was of significant value (Table 2).

Behavioral Characteristics of Baclofen-Selected Animals

As described in the Method section, the distribution of animals between the three subgroups was the following: baclofen responders—22%, baclofen nonresponders—16% and moderate responders—62%. The two extremities (responders and nonresponders) were further characterized using two different models of anxiety. It was found that in elevated plus-maze the % of time spent in open arm and the relation between time spent in and visits into the open arm were significantly lower in baclofen responders (Table 3). When crossings in a special cage between the light and the dark compartment were counted it was found that in baclofen responders the number of crossings was markedly reduced (Table 3). According to these findings novel situations caused comparatively more anxiety in baclofen responders that allows them to be used as a model subpopulation of "anxious" animals.

Central and Peripheral Benzodiazepine Binding Sites in Baclofen-Selected Animals

^{3}H-Flunitrazepam binding in CNS and in peripheral organs was

TABLE 2

^{3}H-FLUNITRAZEPAM BINDING IN NAIVE (STRESSED) AND HANDLING-HABITUATED (UNSTRESSED) RATS CEREBRAL CORTEX AND KIDNEYS

Experimental Group	^{3}H-Flunitrazepam Binding B_{max} (fmol/mg)	%	K_D (nM)	%
	Cerebral Cortex			
Handling-habituated	1123 ± 73	100	1.75 ± 0.13	100
Naive	1472 ± 87*	131	2.12 ± 0.18	121
	Kidneys			
Handling-habituated	5210 ± 283	100	15.6 ± 1.3	100
Naive	5978 ± 239	115	24.1 ± 2.1†	154

In this study tissue membranes washed 4 times with Tris-HCl (pH 7.3) buffer were used. Each value is the mean ± S.E.M. of at least 4 separate experiments. K_D and B_{max} values were determined by Scatchard plot analysis.

*$p<0.05$; †$p<0.01$ as compared to handling-habituated rats.

TABLE 3

COMPARATIVE BEHAVIORAL CHARACTERISTICS OF BACLOFEN RESPONDERS AND NONRESPONDERS IN ELEVATED PLUS-MAZE AND LIGHT/DARK COMPARTMENT TWO-CHAMBERED CAGE MODELS FOR ANXIETY

Parameters Measured	Baclofen Nonresponders (n)	Baclofen Responders (n)
	Elevated Plus-Maze	
% of time spent in open arm	34.5 ± 8.2(9)	11.2 ± 3.6* (9)
Open arm-time: visits	6.5 ± 0.8(8)	2.6 ± 0.6*(10)
	Light/Dark Compartment Cage	
Number of crossings between light and dark compartment	7.4 ± 1.2(9)	3.1 ± 0.6*(11)

The results are expressed as mean ± S.E.M. The number of mice in each group is given in parentheses. *$p<0.05$, significantly different from baclofen nonresponders (Dunnett's t-test).

compared in baclofen responders and nonresponders (Figs. 1 and 2). It was found that the number of binding sites was lower in baclofen responders forebrain and cerebellum (Fig. 1). Similar changes were also observed in peripheral organs like the kidneys and the heart where BD binding sites were labelled using ^{3}H-Ro 5-4864 as a ligand (Fig. 2). The affinity of central and peripheral binding sites did not differ significantly between baclofen responders and nonresponders (data not shown). As mentioned above,

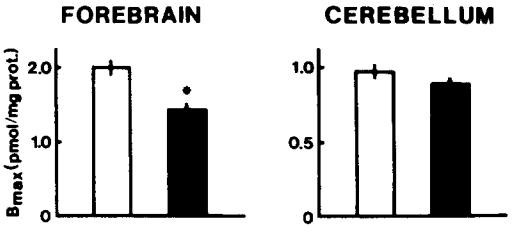

FIG. 1. Maximum binding values of ^{3}H-flunitrazepam binding in unwashed membranes of forebrain and cerebellum in baclofen-selected mice. B_{max} values were found by Scatchard analysis of 4 indpendent experiments. White bars: baclofen nonresponders; black bars: baclofen responders. The data are expressed as mean ± S.E.M. *$p<0.05$ as compared to baclofen nonresponders.

FIG. 2. Maximum binding values of ^{3}H-Ro 5-4864 binding in unwashed membranes of kidney and heart in baclofen-selected mice. B_{max} values were calculated using Scatchard analysis of 4 independent experiments. White bars: baclofen nonresponders; black bars: baclofen responders. The data are expressed as mean ± S.E.M. *$p<0.05$ as compared to baclofen nonresponders.

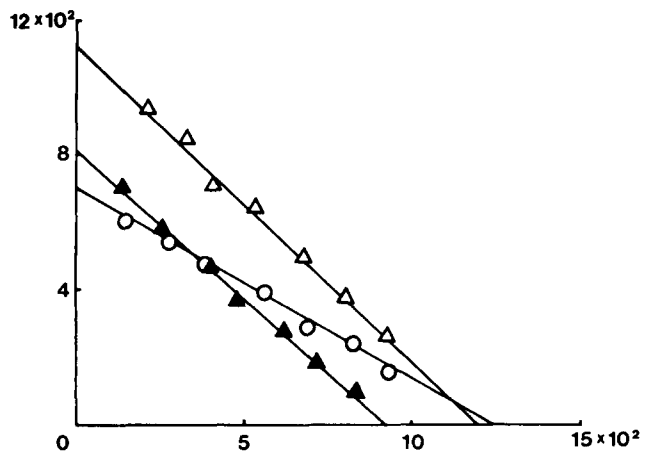

FIG. 3. Scatchard analysis of a typical experiment out of three independent experiments of ^{3}H-flunitrazepam binding to unwashed membranes of rat cerebral cortex: ○: vehicle-, △: muscimol- (1.5 mg/kg) and ▲: (−) baclofen- (5 mg/kg) pretreated animals. Maximum binding and dissociation constant values for ^{3}H-flunitrazepam binding were the following: vehicle: B_{max} 1240 fmol/mg prot., K_D 1.81 nM; muscimol: B_{max} 1180 fmol/mg prot., K_D 1.07 nM; (−)baclofen: B_{max} 918 fmol/mg prot., K_D 1.12 nM. The standard error for the 3 experiments was lower than 3%.

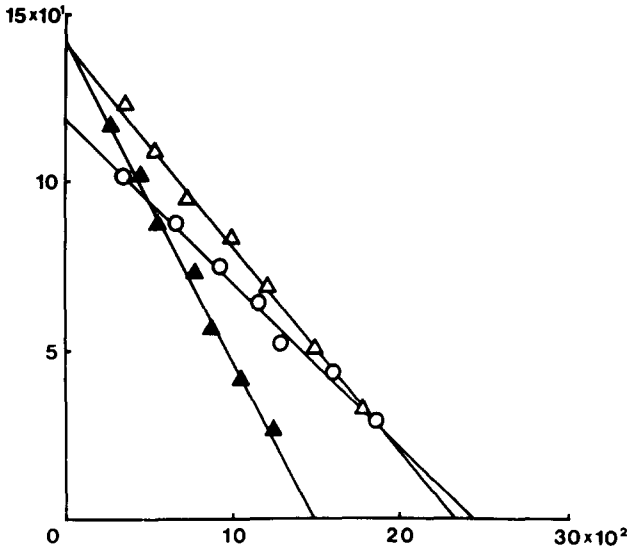

FIG. 4. Scatchard analysis of a typical experiment out of three independent experiments of ^{3}H-flunitrazepam binding to unwashed membranes of rat kidney: ○: vehicle-, △: muscimol- (1.5 mg/kg) and ▲: (−)baclofen- (5 mg/kg) pretreated animals. Maximum binding and dissociation constant values for ^{3}H-flunitrazepam binding were the following: vehicle: B_{max} 2420 fmol/mg prot., K_D 20.5 nM; muscimol: B_{max} 2360 fmol/mg prot., K_D 14.4 nM; (−)baclofen: B_{max} 1490 fmol/mg prot., K_D 12.7 nM. The standard error for the 3 experiments was lower than 3%.

baclofen responders were also diazepam responders and behaviorally characterized as "anxious" animals. In agreement with earlier studies (16,17), "anxious" animals had lower number of BD binding sites in CNS.

The Effect of GABA Agonists on Central and Peripheral BD Binding Sites

In this study the effect of in vivo pretreatment of animals with muscimol (a $GABA_A$ agonist) and (−)baclofen (a $GABA_B$ agonist) on central and peripheral BD binding sites was comparatively studied. Muscimol (1.5 mg/kg) and (−)baclofen (5 mg/kg) increased the affinity of central BD binding sites in cerebral cortex. Interestingly (−)baclofen also lowered the number of BD binding sites in this structure (Fig. 3). The effect of muscimol and baclofen on peripheral BD binding sites in kidneys was similar to the changes observed in cerebral cortex (Fig. 4). In contrast to muscimol, the effect of (−)baclofen on ^{3}H-flunitrazepam binding was more prominent in kidneys

DISCUSSION

In this study we comparatively studied the effect of stressful situations and GABA agonists on central- and peripheral-type BD receptors. It was found that forced swimming immediately increased the number of BD binding sites and parallelly to this lowered their affinity. Our results are in contrast to these of Medina *et al.* (13) who showed biphasic changes in BD binding sites after swimming stress: immediate decrease was followed by an increase. The discrepancy is difficult to explain. However, in our experiments almost three times shorter time of forced swimming was used. Possibly physical exhaustion produced by longer swimming can cause other changes or initial changes produced by stressful situation can change during time course of stressful condition. Additionally to this it should be mentioned that both the increase and the decrease in the number of BD receptors has been reported after stressful manipulations (10,20). Recently, it has been shown that the decapitation procedure itself alters low-affinity GABA receptors in rats (2). As low-affinity GABA receptors are linked to BD receptors we proposed that acute decapitation stress also changes BD receptors. Indeed, we found that in naive (stressed) rats not only the number of BD binding sites was increased but also the affinity was decreased. Although swimming stress can be considered to be more severe, the changes of central BD binding sites in the two models of stress studied were similar.

It is generally agreed that classical $GABA_A$ receptor agonists enhance the affinity of BD receptors in vivo and in vitro, whereas diazepam increases the binding of tritiated agonists to $GABA_A$ receptors indicating the existence of coupling between $GABA_A$ and BD receptors (5). On the contrary, relatively little is known about the interaction of $GABA_B$ recognition sites and BD receptors. Our present results indicating that in vivo pretreatment of animals with muscimol increases the affinity of BD receptors are in agreement with previous data (21). The results showing that in vivo administration of (−)baclofen not only increases the affinity of central BD receptors, but also decreases the number of binding sites are in contrast to the previous results obtained in vitro demonstrating the absence of coupling between $GABA_B$ and BD receptors (4). However, previously it has also been shown that in vivo baclofen can alter BD binding (6). Furthermore, recently we found that another $GABA_B$ receptor agonist fenibut (β-phenyl-GABA), can also alter BD binding (18). As in our experiments, stressful conditions lowered the affinity and increased maximum binding of BD ligands, we suggest that both GABA receptor agonists may have a stress protective effect on the level of BD receptors.

In baclofen-selected animals it was shown that BD binding is lower in baclofen responders as compared to baclofen nonresponders. This subgroup of mice was also found to be behaviorally more "anxious" than baclofen nonresponders. These data are in agreement with the data of others, who have shown that "anxious" animals have a lower density of central BD receptors (16,17).

As in all experiments described here the changes in peripheral

BD binding sites were similar to those of central ones the question arises how stress and GABA agonists can regulate peripheral BD receptors. Several studies have ascertained that there are no ^{3}H-muscimol binding sites, no GABAergic regulation, no barbiturate regulation and no ^{35}S-TBPS binding present in the purified peripheral type BD receptors (12). Although the problem on endogeneous ligand for BD receptors is still unsolved several groups have proposed the existence of such kind of compounds [for review see (8)]. Our present data about similar regulation of central and peripheral BD binding sites by stress and GABA agonists seem to support the idea that there should be at least one common endogeneous modulator (ligand?) for both types of BD receptors. The localization of relatively high concentrations of peripheral BD binding sites in many endocrinologically important organs like adrenals, pituitary and pineal gland (1,9) may indicate on the possibility that these binding sites could be involved in the neuroendocrinological regulation of emotional and stress reactions in response to environmental conditions. In this respect our data presented here are indirectly supported by the experiments of M. Gavish *et al.* (7) who demonstrated that hyperthyroidism produced by chronical treatment with T_4 produced elevation of both central and peripheral BD binding sites. There is no doubt that hyperthyroidism can also cause changes in emotional behavior.

In conclusion, from BD binding studies carried out here, it is possible to assume that central- and peripheral-type BD receptors are regulated similarly by stress and GABA agonists. Although the physiological mechanisms involved in the similar regulation of peripheral BD sites remain to be elucidated we would like to indicate on a possibility to use peripheral BD binding sites (on blood platelets for example) as sensitive markers of functional activity of central BD/GABA receptoral complex.

REFERENCES

1. Anholt, R. R. H. Mitochondrial benzodiazepine receptor as potential modulators of intermediary metabolism. Trends Pharmacol. Sci. 7:506–511; 1986.
2. Biggio, G. The action of stress, beta-carbolines, diazepam and Ro 15-1788 on GABA receptors in the rat brain. In: Biggio, G.; Costa, E., eds. Benzodiazepine recognition site ligands: Biochemistry and pharmacology. New York: Raven Press; 1983:400–403.
3. Blumstein, L. K.; Crawley, J. N. Further characterization of a simple, automated exploratory model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 18:37–40; 1983.
4. Bowery, N. G.; Hill, D. R.; Hudson, A. L.; Price, G. W.; Turnbull, M. J.; Wilkin, G. W. Heterogeneity of mammalian GABA receptors. In: Bowery, N. G., ed. Actions of GABA and benzodiazepines. New York: Raven Press; 1984:81–108.
5. Costa, E.; Guidotti, A. Molecular mechanism in the receptor action of benzodiazepines. Annu. Rev. Pharmacol. Toxicol. 19:531–545; 1979.
6. Gallager, W. D.; Thomas, J. W.; Tallman, J. F. Effect of GABA-ergic drugs on benzodiazepine binding site sensitivity in rat cerebral cortex. Biochem. Pharmacol. 27:2745–2749; 1978.
7. Gavish, M.; Weitzman, A.; Okun, F.; Youdin, M. B. H. Modulatory effects of thyroxine treatment on central and peripheral benzodiazepine receptors in the rat. J. Neurochem. 47:1106–1110; 1986.
8. Haefely, W. Endogenous ligands of the benzodiazepine receptor. Pharmacopsychiatry 21:43–46; 1988.
9. Le Fur, G.; Perrier, M. L.; Vancher, N.; Imbault, F.; Flamier, A.; Benavides, J.; Uzan, A.; Renault, C.; Dubroencq, M. C.; Gueremy, C. Peripheral benzodiazepine binding sites: Effect of PK 11195, 1-(2-chloro-phenyl)-N-methyl-(1-methylpropyl)-3-isoquinolinecarboxamide. I. In vitro studies. Life Sci. 32:1839–1847; 1983.
10. Lippa, A. S.; Klefner, C. A.; Yunger, L.; Sano, M. C.; Smith, W. V.; Beer, B. Relationship between benzodiazepine receptors and experimental anxiety in rats. Pharmacol. Biochem. Behav. 9:853–856; 1978.
11. Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275; 1951.
12. Martini, C.; Lucacchini, A.; Hrelia, S.; Rossi, C. A. Central- and peripheral-type benzodiazepine receptors. In: Biggio, G.; Costa, E., eds. GABA-ergic transmission and anxiety. New York: Raven Press; 1986:1–11.
13. Medina, J. H.; Novas, M. L.; Wolfman, C. N. Benzodiazepine receptors in rat cerebral cortex and hippocampus undergo rapid and reversible changes after acute stress. Neuroscience 9:331–335; 1983.
14. Patel, J.; Marangos, P. J. Differential effects of GABA on peripheral and central type benzodiazepine binding sites in brain. Neurosci. Lett. 30:157–160; 1982.
15. Pellow, S.; File, S. E. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24:525–529; 1986.
16. Robertson, H. A.; Martin, I. L.; Candy, J. M. Differences in benzodiazepine receptor binding: Maudsley reactive and Maudsley non-reactive rats. Eur. J. Pharmacol. 50:455–457; 1978.
17. Robertson, H. A. Benzodiazepine receptors in "emotional" and "non-emotional" mice: comparison of four strains. Eur. J. Pharmacol. 56:163–166; 1979.
18. Rägo, L. K.; Kiivet, R.-A. K.; Harro, J. E.; Allikmets, L. H. Benzodiazepine binding sites in mice forebrain and kidneys: Evidence for similar regulation by GABA agonists. Pharmacol. Biochem. Behav. 24:1–3; 1986.
19. Rägo, L. K.; Kiivet, R.-A. K.; Harro, J. E. Variation in behavioral response to baclofen: correlation with benzodiazepine binding sites in mouse forebrain. Naunyn Schmiedebergs Arch. Pharmacol. 333: 303–306; 1986.
20. Soubrie, P.; Thicbot, M. H.; Jobert, A.; Montrastruc, S. L.; Hery, F.; Hemon, M. Decreased convulsant potency of picrotoxin and pentylenetetrazol and enhanced ^{3}H-flunitrazepam cortical binding following stressful manipulations in rats. Brain Res. 189:505–517; 1980.
21. Tallman, J. F.; Thomas, J. W.; Gallager, D. W. GABA-ergic modulation of benzodiazepine binding site sensitivity. Nature 274: 383–385; 1978.