Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 2, pp. 249–254, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00422-6

Antagonism of Isoniazid-Induced Convulsions by Abecarnil in Mice Tolerant to Diazepam

MARIANGELA SERRA,[1] CRISTINA A. GHIANI, COSTANTINO MOTZO, MARIA LUISA PORCEDDU AND GIOVANNI BIGGIO

Department of Experimental Biology, University of Cagliari, Italy

Received 19 September 1994; Revised 14 November 1994; Accepted 14 November 1994

SERRA, M., C. A. GHIANI, C. MOTZO, M. L. PORCEDDU AND G. BIGGIO. *Antagonism of isoniazid-induced convulsions by abecarnil in mice tolerant to diazepam.* PHARMACOL BIOCHEM BEHAV **52**(2) 249–254, 1995.—The ability of the benzodiazepine receptor full agonist diazepam, the selective agonist abecarnil, and the partial agonist imidazenil to antagonize convulsions induced by isoniazid (200 mg/kg, SC) was studied in mice chronically treated with diazepam (3 mg/kg, IP, three times daily) or abecarnil (0.1 or 1 mg/kg, IP, three times daily or 6 mg/kg, SC, daily). Diazepam induced tolerance to its own anticonvulsant effect. In contrast, chronic treatment with abecarnil failed to induce tolerance to its own anticonvulsant activity. Animals treated with abecarnil at 0.1 mg/kg developed cross-tolerance to imidazenil, whereas those treated with 1 mg/kg became less sensitive to diazepam. Mice chronically treated with abecarnil at 6 mg/kg showed almost complete tolerance to diazepam. Abecarnil was able to antagonize the convulsions elicited by isoniazid in diazepam-tolerant mice. These data indicate that chronic administration of abecarnil, unlike that of classical benzodiazepines, does not induce tolerance to its anticonvulsant effect, and that abecarnil overcomes tolerance induced by long-term treatment with the full agonist diazepam.

Abecarnil Imidazenil Diazepam Chronic treatment Tolerance Cross-tolerance Convulsions Mouse

ALTHOUGH benzodiazepines are effective antiepileptic drugs, tolerance to their anticonvulsant actions can limit the long-term effectiveness of these compounds (2,9,21). Recent studies of the pharmacology of γ-aminobutyric acid type A ($GABA_A$) receptors have shown that benzodiazepine receptor partial agonists, as well as ligands that possess higher affinity or efficacy at specific subpopulations of $GABA_A$ receptors, fail to induce tolerance to their anticonvulsant effects (6,8, 14,19). Such compounds may therefore mantain their pharmacological and therapeutic efficacy during long-term treatment. The β-carboline abecarnil and the imidazobenzodiazepine imidazenil, two new anxioselective and anticonvulsant allosteric modulators of $GABA_A$ receptors, possess marked anticonvulsant activity with low tolerance liabilities (6,7,15,22,23,26). Although the molecular mechanisms responsible for the actions of abecarnil and imidazenil are not known in detail, the ability of these drugs to induce fewer side effects at pharmacologically effective doses may be attributable to a preferential affinity for and high efficacy at specific $GABA_A$ receptor subtypes in the case of abecarnil (10,20) and the partial agonist profile of imidazenil (7).

We have now evaluated the development of tolerance to the anticonvulsant effect of abecarnil and cross-tolerance to the effects of imidazenil and diazepam after long-term treatment of mice with abecarnil. We also investigated whether long-term treatment with diazepam induces cross-tolerance to abecarnil and imidazenil.

METHOD

Animals

Male CD-1 mice (Charles River, Como, Italy) with body masses of 25–30 g were mantained under a 12L : 12D cycle at a temperature of 23 ± 2°C and 65% humidity. After arrival at the animal facility, the mice were acclimatized for ≥ 7 days, during which they had free access to food and water.

[1] Requests for reprints should be addressed to Mariangela Serra, Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, 09123 Cagliari, Italy.

TABLE 1

EFFECT OF ACUTE ADMINISTRATION OF IMIDAZENIL, ABECARNIL, AND DIAZEPAM ON ISONIAZID-TREATED MICE

Experimental group (mg/kg)	Convulsions		Death	
	Latency (min)	No. of Animals	Latency (min)	No. of Animals
Isoniazid (200)	55 ± 7.5	49/50	67 ± 6	43/50
Isoniazid + imidazenil (0.1)	88 ± 5.9*	18/42†	97 ± 9*	12/42†
Isoniazid + imidazenil (1)	88 ± 10*	7/30†	91 ± 4*	4/30†
Isoniazid + abecarnil (0.1)		0/15†		0/15†
Isoniazid + diazepam (3)		0/15†		0/15†

Animals were observed for 4 h, during which the latency of tonic-clonic seizures and death was recorded. Values are means ± SEM of three to five separate experiments.

*$p < 0.01$ vs. isoniazid-treated mice (Student's t-test).

†$p < 0.05$ vs. isoniazid-treated mice (Fisher's exact probability test).

Treatments

Abecarnil (0.1 or 1 mg/kg, IP) or diazepam (3 mg/kg, IP) was administered three times daily (0800, 1400, 2000 h) for 30 days. The drugs were suspended in distilled water with a drop of Tween-80 per 5 ml and injected in a volume of 10 ml per kilogram of body mass. Control mice received an equivalent volume of vehicle.

A parallel group of mice was treated subcutaneously once a day for 15 days with abecarnil (6 mg/kg) dissolved in sesame oil and administered in a volume of 4 ml/kg. Control mice received an equivalent volume of vehicle.

Isoniazid was dissolved in distilled water and administered (200 mg/kg, SC) in a volume of 10 ml/kg; control mice received an equivalent volume of vehicle. Animals received the challenge injection (IP) of abecarnil, diazepam, or imidazenil 36 or 72 h after the last chronic administration and 10 min after isoniazid. All animals were observed for the appearance of convulsions for ≥ 4 h; the time of onset of seizure activity was recorded and the pattern of the seizures was noted.

Statistics

Data were analyzed by Fisher's exact probability test or Student's t-test.

Chemicals

Imidazenil was provided by Fidia Research Laboratories (Abano Terme, Italy), abecarnil was a gift of Schering AG

TABLE 2

EFFECT OF LONG-TERM TREATMENT WITH A LOW DOSE (0.1 mg/kg) OF ABECARNIL ON THE DEVELOPMENT OF TOLERANCE TO THE ANTICONVULSANT ACTIONS OF ABECARNIL,IMIDAZENIL, AND DIAZEPAM

Experimental Group (mg/kg)	Convulsions		Death	
	Latency (min)	No. of Animals	Latency (min)	No. of Animals
Chronic vehicle				
Isoniazid (200)	48 ± 2	45/45	63 ± 2.6	43/45
Isoniazid + abecarnil (0.1)	78 ± 4*	2/18†	83*	1/18†
Isoniazid + imidazenil (0.1)	76 ± 7*	9/25†	103 ± 8*	3/25†
Isoniazid + diazepam (3)		0/15†		0/15†
Chronic abecarnil				
Isoniazid (200)	43 ± 4	65/65	58 ± 2.3	65/65
Isoniazid + abecarnil (0.1)	75 ± 7‡	6/24§	78 ± 1.4‡	3/24§
Isoniazid + imidazenil (0.1)	67 ± 3‡	31/36	83 ± 7‡	21/36§
Isoniazid + diazepam (3)	80 ± 2‡	2/15§		0/15§

Mice were treated with abecarnil (0.1 mg/kg) or vehicle three times a day for 30 days. Isoniazid and the challenge injections were administered 36 h after the last chronic treatment. Animals were observed for 4 h, during which the latency of tonic-clonic convulsions and death was recorded. Values are means ± SEM of three separate experiments.

*$p < 0.01$ vs. chronic vehicle + isoniazid; (Student's t-test).

†$p < 0.025$ vs. chronic vehicle + isoniazid; (Fisher's exact probability test).

‡$p < 0.01$ vs. chronic abecarnil + isoniazid (Student's t-test).

§$p < 0.01$ vs. chronic abecarnil + isoniazid (Fisher's exact probability test).

TABLE 3

EFFECT OF LONG-TERM TREATMENT WITH A HIGH DOSE (1 mg/kg) OF ABECARNIL ON THE DEVELOPMENT OF TOLERANCE TO THE ANTICONVULSANT ACTIONS OF ABECARNIL AND DIAZEPAM

	Convulsions		Death	
Experimental Group (mg/kg)	Latency (min)	No.of Animals	Latency (min)	No. of Animals
Chronic vehicle				
Isoniazid (200)	47 ± 1	30/30	61 ± 2	30/30
Isoniazid + abecarnil (1)		0/10*		0/10*
Isoniazid + diazepam (3)	115†	1/15*		0/15*
Chronic abecarnil				
Isoniazid (200)	40 ± 4	35/35	63 ± 3	33/35
Isoniazid + abecarnil (1)	73 ± 2‡	2/25§	100‡	1/25§
Isoniazid + diazepam (3)	80 ± 5‡	21/32§	105 ± 2‡	19/32§

Mice were treated with abecarnil (1 mg/kg) or vehicle three times a day for 30 days. Isoniazid and the challenge injections were administered 36 h after the last chronic treatment. Animals were observed for 4 h, during which the latency of tonic-clonic convulsions and death was recorded. Values are means ± SEM of three separate experiments.

*$p < 0.01$ vs. chronic vehicle + isoniazid; (Fisher's exact probability test).
†$p < 0.01$ vs. chronic vehicle + isoniazid; (Student's t-test).
‡$p < 0.01$ vs. chronic abecarnil + isoniazid (Student's t-test).
§$p < 0.025$ vs. chronic abecarnil + isoniazid (Fisher's exact probability test).

(Berlin, Germany), and diazepam was a gift from Hoffmann-La Roche (Basel, Switzerland). Isoniazid (isonicotinic acid hydrazide) was obtained from Sigma (St. Louis, MO). Other drugs and materials were obtained from commercial sources.

RESULTS

Acute studies

Acute administration of abecarnil, imidazenil, or diazepam antagonized isoniazid-induced convulsions in a dose-depen-

TABLE 4

EFFECT OF LONG-TERM TREATMENT WITH ABECARNIL (6 mg/kg) ADMINISTERED SUBCUTANEOUSLY ON THE DEVELOPMENT OF TOLERANCE TO THE ANTICONVULSANT ACTIONS OF ABECARNIL, IMIDAZENIL, AND DIAZEPAM

	Convulsions		Death	
Experimental Group (mg/kg)	Latency (min)	No. of Animals	Latency (min)	No. of Animals
Chronic vehicle				
Isoniazid (200)	44 ± 2	27/27	53 ± 3	27/27
Isoniazid + abecarnil (0.1)		0/18*		0/18*
Isoniazid + imidazenil (1)	89 ± 4†	5/18*	98 ± 5†	2/18*
Isoniazid + diazepam (3)		0/18*		0/18*
Chronic abecarnil				
Isoniazid (200)	37 ± 6	36/36	55 ± 6	36/36
Isoniazid + abecarnil (0.1)	82 ± 3§	5/21‡	94 ± 5§	2/21‡
Isoniazid + imidazenil (1)	47 ± 2	21/21	67 ± 5§	18/21
Isoniazid + diazepam (3)	66 ± 6§	21/28	78 ± 5§	5/21‡

Mice were treated with abecarnil (6 mg/kg, SC) or vehicle once a day for 15 days. Isoniazid and the challenge injections were administered 72 h after the last chronic treatment. Animals were observed for 4 h, during which the latency of tonic-clonic convulsions and death was recorded. Values are means ± SEM of three separate experiments.

*$p < 0.01$ vs. chronic vehicle + isoniazid; (Fisher's exact probability test).
†$p < 0.01$ vs. chronic vehicle + isoniazid; (Student's t-test).
‡$p < 0.01$ vs. chronic abecarnil + isoniazid (Fisher's exact probability test).
§$p < 0.01$ vs. chronic abecarnil + isoniazid (Student's t-test).

TABLE 5

LONG-TERM TREATMENT WITH DIAZEPAM (3 mg/kg) ON THE DEVELOPMENT OF TOLERANCE TO THE ANTICONVULSANT ACTIONS OF DIAZEPAM, ABECARNIL, AND IMIDAZENIL

	Convulsions		Death	
Experimental Group (mg/kg)	Latency (min)	No. of Animals	Latency (min)	No. of Animals
Chronic vehicle				
Isoniazid (200)	51 ± 2.5	20/20	67 ± 4	19/20
Isoniazid + diazepam (3)		0/15*		0/15*
Isoniazid + abecarnil (1)		0/15*		0/15*
Isoniazid + imidazenil (1)	88 ± 7†	3/15*	92 ± 4†	2/15*
Chronic diazepam				
Isoniazid (200)	36 ± 2	28/28	56 ± 3	27/28
Isoniazid + diazepam (3)	63 ± 6‡	13/15	98 ± 9‡	5/15§
Isoniazid + abecarnil (1)	80 ± 6‡	5/15§	82 ± 1‡	1/15§
Isoniazid + imidazenil (1)	58 ± 3‡	15/15	82 ± 6‡	13/15

Mice were treated with diazepam (3 mg/kg) or vehicle three times a day for 30 days. Isoniazid and the challenge injections were administered 36 h after the last chronic treatment. Animals were observed for 4 h, during which the latency of tonic-clonic convulsions and death was recorded. Values are means ± SEM of three separate experiments.

*$p < 0.01$ vs. chronic vehicle + isoniazid; (Fisher's exact probability test).
†$p < 0.01$ vs. chronic vehicle + isoniazid; (Student's t-test).
‡$p < 0.01$ vs. chronic diazepam + isoniazid (Student's t-test).
§$p < 0.01$ vs. chronic diazepam + isoniazid (Fisher's exact probability test).

dent manner (Table 1). At a dose of 0.1 mg/kg (IP), imidazenil reduced by 57% the number of animals that exhibited seizures or died, respectively, and significantly prolonged the time before onset of convulsions or death in isoniazid-treated mice. At a higher dose (1 mg/kg, IP) the effects of imidazenil were more marked. Abecarnil was more potent and efficacious than imidazenil in antagonizing isoniazid-induced convulsions; at a dose of 0.05 mg/kg (IP), abecarnil decreased by 70% the number of animals that exibited seizures (22), and at 0.1 mg/kg (IP) the drug completely prevented seizure activity (Table 1). A higher dose of diazepam (3 mg/kg, IP) also abolished seizure activity.

Chronic Administration of Abecarnil

As expected (15), long-term treatment with abecarnil (0.1 or 1 mg/kg, IP; three times daily) failed to induce tolerance to the anticonvulsant effect of this drug in mice (Tables 2 and 3). Challenge doses (0.1 or 1 mg/kg, IP, respectively) of abecarnil administered 36 h after the last injection of the respective chronic treatment showed similar efficacies in antagonizing isoniazid-induced convulsions and death in mice chronically treated with abecarnil and in control mice.

Mice chronically treated with abecarnil at 0.1 mg/kg developed cross-tolerance to the anticonvulsant action of imidazenil (0.1 mg/kg, IP) (Table 2). Diazepam (3 mg/kg, IP) showed similar anticonvulsant efficacies in control animals and mice chronically treated with abecarnil at 0.1 mg/kg. However, the anticonvulsant efficacy of diazepam was markedly decreased in mice chronically treated with abecarnil at 1 mg/kg (Table 3).

It has been reported that abecarnil does not form active metabolites, and at doses of 0.1–5 mg/kg has a short half-life (1 h) (11). To investigate whether the failure of long-term abecarnil treatment to induce tolerance was attributable to the pharmacokinetics of the drug, we injected subcutaneously a separate group of mice once a day for 15 days with abecarnil (6 mg/kg). Under these conditions, the occupancy of benzodiazepine receptors in the mouse forebrain decreased from 83% at 7 h to 31% at 24 h after injection (25). Three days after the last chronic treatment, when the benzodiazepine receptor occupancy is only 13% (25), mice were injected with isoniazid and the challenge drugs. Consistent with the result of the repeated intraperitoneal treatment protocol, the abecarnil-treated mice failed to develop tolerance to the anticonvulsant effect of abecarnil but did develop cross-tolerance to the anticonvulsant effects of both imidazenil (1 mg/kg) and diazepam (3 mg/kg) (Table 4).

Chronic Administration of Diazepam

As expected (3,4,21), repeated administrations of diazepam induced tolerance to the anticonvulsant effect of this drug (Table 5). Mice tolerant to diazepam exhibited cross-tolerance to the anticonvulsant action of imidazenil (1 mg/kg) but not to that of abecarnil (1 mg/kg).

DISCUSSION

Chronic administration of classical benzodiazepines in rodents results in both a decrease in $GABA_A$ receptor function and the development of tolerance to most of the pharmacological effects of these drugs (5,13,17,21). We have now shown that long-term treatment with diazepam, a full agonist of the benzodiazepine receptor, does not induce tolerance to the anticonvulsant effect of abecarnil, and that long-term treatment with abecarnil does not induce tolerance to the anticonvulsant effect of this drug. Thus, challenge doses (0.1 or 1 mg/kg,

respectively) of abecarnil antagonized with similar efficacies the tonic-clonic seizures elicited by isoniazid in mice chronically treated with abecarnil (0.1 or 1 mg/kg, respectively) and in control animals. The lowest challenge dose (0.1 mg/kg) of abecarnil was less effective in mice chronically treated with vehicle than in naive animals. A decreased efficacy of abecarnil in animals chronically treated with vehicle was also observed with regard to the effect of this drug on *t*-[^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS) binding to cortical membranes (24). Repeated handling during chronic vehicle treatment may account for this decreased efficacy; this conclusion is consistent with evidence that handling habituation reduces the effect of anxiolytic drugs (1,3). Our observation that abecarnil did not induce anticonvulsant tolerance after chronic treatment in mice is consistent with the results of previous studies showing little pharmacological tolerance to the anticonvulsant effect of abecarnil in dogs and rats (14,15).

Although abecarnil was able to antagonize the convulsions elicited by isoniazid in diazepam-tolerant mice, animals chronically treated with abecarnil, in which abecarnil mantained its pharmacological action, developed cross-tolerance to both the partial agonist imidazenil and the full agonist diazepam. Thus, imidazenil failed to antagonize isoniazid-induced convulsions in mice chronically treated with the lowest dose (0.1 mg/kg, IP) of abecarnil, whereas the anticonvulsant efficacy of diazepam decreased as the dose of chronic abecarnil treatment increased; diazepam (3 mg/kg) failed to prevent isoniazid-induced seizures in all animals treated with abecarnil at 6 mg/kg (SC).

Recent pharmacological and molecular studies have demonstrated that abecarnil and diazepam exhibit qualitatively different receptor properties. Thus, abecarnil shows less efficacy at $GABA_A$ receptors containing α_5 and α_1 subunits than at receptors containing the α_3 subunit (10,20), and receptors containing α_4 or α_6 subunits are insentive to diazepam (16,27). Moreover, variants of the γ subunit can influence both affinity and efficacy of diazepam and abecarnil (both are higher in the presence of γ_2 vs. γ_1) (26). On the basis of these data, one may speculate that abecarnil acts as a full agonist at populations of receptors that are also sensitive to diazepam and as a partial agonist at other receptor populations that are diazepam insensitive. Thus, chronic treatment with abecarnil would downregulate those receptors at which it acts as a full agonist, but not those at which it acts as a partial agonist. The result of such selective receptor interaction might be the development of differential tolerance to diazepam and abecarnil in animals chronically treated with these drugs.

An alternative explanation of our data is that chronic abecarnil treatment might modulate the expression of $GABA_A$ receptor subunit genes in a manner that favors the assembly of receptors with reduced sensitivity to diazepam but not to abecarnil. Changes in the abundance of messengers RNAs that encode various $GABA_A$ receptor subunits have been demonstrated after chronic treatment with benzodiazepine receptor ligands (12,18).

In conclusion, we have shown that long-term treatment with abecarnil did not induce tolerance to the anticonvulsant effect of this drug, and that mice chronically treated with diazepam did not develop cross-tolerance to abecarnil. Should these observations be reproduced at the clinical level, abecarnil may prove a useful therapeutic agent with which to treat patients who are tolerant to the anticonvulsant effect of classical benzodiazepine full agonists.

REFERENCES

1. Boix, F.; Fernandez Teruel, A.; Tobena, A. The anxiolytic action of benzodiazepines is not present in handling habituated rats. Pharmacol. Biochem. Behav. 31:541–546; 1989.
2. File, S. E. Tolerance to the anti-pentylentetrazole effects of diazepam in the mouse. Psychopharmacology (Berlin) 79:284–286; 1983.
3. File, S. E.; Andrews, N.; Wu, P. Y.; Zharkovsky, A.; Zangrossi, H., Jr. Modification of chlordiazepoxide's behavioural and neurochemical effects by handling and plus-maze experience. Eur. J. Pharmacol. 218:9–14; 1992.
4. Frey, H. H.; Philippin, H. P.; Scheuler, W. Development of tolerance to the anticonvulsant effect of diazepam in dogs. Eur. J. Pharmacol. 104:27–32; 1984.
5. Gallager, D. W.; Marley, R. J.; Hernandez, T. D. Biochemical and electrophysiological mechanisms underlying benzodiazepine tolerance and dependence. In: Pratt, J., ed. The biological bases of drug tolerance and dependence. London: Academic Press; 1991:49–70.
6. Ghiani, C. A.; Serra, M.; Motzo, C.; Giusti, P.; Cuccheddu, T.; Porceddu, M. L.; Biggio, G. Chronic administration of an anticonvulsant dose of imidazenil fails toinduce tolerance of $GABA_A$ receptor function in mice. Eur. J. Pharmacol. 254:299–302; 1994.
7. Giusti, P.; Ducic, I.; Puia, G.; Arban, R.; Walser, A.; Guidotti, A.; Costa, E. Imidazenil: A new partial positive allosteric modulator of GABA action at $GABA_A$ receptors. J. Pharmacol. Exp. Ther. 266:1018–1028; 1993.
8. Haefely, W. E.; Martin, J. R.; Schoch, P. Novel anxiolytics that act as partial agonists at benzodiazepine receptors. Trend Pharmacol. Sci. 11:452–456; 1990.
9. Haigh, J. R.; Feely, M. Ro 16-6028, a benzodiazepine receptor partial agonist, does not exhibit anticonvulsant tolerance in mice. Eur. J. Pharmacol. 147:282–285; 1988.
10. Knoflach, F.; Drescher, V.; Schenzer, L.; Malherbe, P.; Mohler, H. Full and partial agonism displayed by benzodiazepine receptor ligands at recombinant γ-aminobutyric $acid_A$ receptor subtypes. J. Pharmacol. Exp. Ther. 266:385–391; 1993.
11. Krause, W.; Mengel, H. Pharmacokinetics of the anxiolytic β-carboline derivative abecarnil in the mouse, rat, rabbit, dog, cynomolgus monkey and baboon. Arzneimforschung Drug Res. 40: 522–529; 1990.
12. Lewin, E.; Bleck, V.; Dildy-Mayfield, J. E.; Harris, R. A. $GABA_A$ and glutamate receptor subunit mRNAs in cortex of mice chemically kindled with FG 7142. Mol. Brain Res. 22:320–322; 1994.
13. Lister, R. G.; File, S. E.; Greenblatt, D. J. Functional tolerance to lorazepam in the rat. Psychopharmacology (Berlin) 81:292–294; 1983.
14. Löscher, W.; Hönack, D.; Scherkl, R.; Hashem, A.; Frey, H. H. Pharmacokinetics,anticonvulsant efficacy and adverse effects of the β-carboline abecarnil, a novel ligand for benzodiazepine receptors, after acute and chronic administration in dogs. J. Pharmacol. Exp. Ther. 255:541–548; 1990.
15. Löscher, W.; Rundfeldt, C.; Hönack, D. Tolerance to anticonvulsant effects of the partial benzodiazepine receptor agonist abecarnil in kindled rats involves learning. Eur. J. Pharmacol. 202: 303–310; 1991.
16. Luddens, H.; Pritchett, D. B.; Kohler, M.; Killisch, I.; Keinamen, K.; Monyer, H.; Sprengel, R.; Seeburg, P. H. Cerebellar $GABA_A$ receptor selective for a behavioural alcohol antagonist. Nature 346:648–651; 1990.
17. Miller, L. G.; Greenblatt, D. J.; Barnhill, J. G.; Shader, R. I. Chronic benzodiazepine administration. I. Tolerance is associated with benzodiazepine receptor downregulation and decreased γ-aminobutyric $acid_A$ receptor function. J. Pharmacol. Exp. Ther. 264:170–176; 1988.

18. O'Donovan, M. C.; Buckland, P. R.; Spurlook, G.; McGuffin, P. Bidirectional changes in the levels of messenger RNAs encoding γ-aminobutyric $acid_A$ receptor α subunits after flurazepam treatment. Eur. J. Pharmacol. Mol. Pharmacol. 226:335–341; 1992.
19. Perrault, G.; Morel, E.; Sanger, D. J.; Zivkovic, B. Lack of tolerance and physical dependence upon repeated treatment with the novel hypnotic zolpidem. J. Pharmacol. Exp. Ther. 263:298–303; 1992.
20. Pribilla, I.; Neuhaus, R.; Hillman, M.; Turner, J.; Stephens, D. N.; Schneider, H. H. Abecarnil is a full agonist at some, and a partial agonist at other recombinant $GABA_A$ receptor subtypes. In: Stephens, D. N., ed. Anxiolytic β-carbolines: From molecular biology to the clinic. Heidelberg, Germany: Springer-Verlag; 1993:50–61.
21. Rosenberg, H. C.; Tietz, E. I.; Chiu, T. H. Tolerance to the anticonvulsant action of benzodiazepines. Neuropharmacology 24:639–644; 1985.
22. Serra, M.; Foddi, M. C.; Ghiani, C. A.; Melis, M. A.; Motzo, C.; Concas, A.; Sanna, E.; Biggio, G. Pharmacology of $GABA_A$ receptor complex after the in vivo administration of the anxioselective and anticonvulsant β-carboline abecarnil. J. Pharmacol. Exp. Ther. 263:1360–1368; 1992.
23. Serra, M.; Ghiani, C. A.; Motzo, C.; Cuccheddu, T.; Floris, S.; Giusti, P.; Biggio, G. Imidazenil, a new partial agonist at benzodiazepine receptors, reverses the inhibitory action of isoniazid and stress on γ-aminobutyric $acid_A$ receptor function. J. Pharmacol. Exp. Ther. 269:32–38; 1994.
24. Serra, M.; Ghiani, C. A.; Motzo, C.; Porceddu, M. L.; Biggio, G. Long-term treatment with abecarnil fails to induce tolerance in mice. Eur. J. Pharmacol. 259:1–6; 1994.
25. Steppuhn, K. G.; Schneider, H. H.; Turski, L.; Stephens, D. N. Long-term treatment with abecarnil does not induce diazepam-like dependence in mice. J. Pharmacol. Exp. Ther. 264:1395–1400; 1993.
26. Turski, L.; Stephens, D. N.; Jensen, L. H.; Petersen, E. N.; Meldrum, B. S.; Patel, S.; Bondo Hansen, J.; Löscher, W.; Schneider, H. H.; Schmiechen, R. Anticonvulsant action of the β-carboline abecarnil: Studies in rodents and baboon, *Papio papio*. J. Pharmacol. Exp. Ther. 253:344–352; 1990.
26. Wafford, K. A.; Boin, C. J.; Whiting, P. J.; Kemp, J. A. Functional comparison of the role of γ-subunits in recombinant human γ-aminobutyric $acid_A$/benzodiazepine receptors. Mol. Pharmacol. 44:437–442; 1993.
27. Wisden, W.; Herb, A.; Wieland, H.; Keinänem, K.; Lüddens, H.; Seeburg, P. H. Cloning, pharmacological characteristics and expression pattern of the rat $GABA_A$ receptor α_4 subunit. FEBS Lett. 282:227–230; 1991.