Biochemical Pharmacology, Vol. 42, Suppl., pp. S99–S104, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

CHRONIC BENZODIAZEPINE ADMINISTRATION

IX. ATTENUATION OF ALPRAZOLAM DISCONTINUATION EFFECTS BY CARBAMAZEPINE

WENDY R. GALPERN, LAWRENCE G. MILLER,* DAVID J. GREENBLATT, GEORGE K. SZABO, THOMAS R. BROWNE and RICHARD I. SHADER

Division of Clinical Pharmacology, Departments of Psychiatry and Pharmacology, Tufts University School of Medicine and New England Medical Center, Boston, MA; and the Neuropharmacology Laboratory, Department of Neurology, Boston University School of Medicine and Boston Veterans Affairs Medical Center, Boston, MA, U.S.A.

(*Received* 12 *April* 1991; *accepted* 19 *August* 1991)

Abstract—Clinical studies suggest that carbamazepine may attenuate effects of alprazolam discontinuation. Since discontinuation of chronic alprazolam in a mouse model is associated with behavioral alterations and upregulation at the γ-aminobutyric acid$_A$ ($GABA_A$) receptor, we studied the effects of carbamazepine administration after alprazolam (2 mg/kg/day) discontinuation. Open-field activity was increased in mice 4 days after alprazolam discontinuation, but this effect was reduced significantly by continuous infusion of carbamazepine, 25 or 100 mg/kg/day. Benzodiazepine receptor binding *in vivo* was increased in cortex at 2 and 4 days after alprazolam discontinuation, and in hypothalamus at 4 days; with carbamazepine, 100 mg/kg/day, binding in both regions at these time points was similar to control values. Similar results were observed in cortex with benzodiazepine receptor binding *in vitro*. GABA-dependent chloride uptake was also increased at 4 days after alprazolam administration. Treatment with carbamazepine attenuated ($P < 0.10$) this increase. Carbamazepine alone after vehicle did not alter benzodiazepine binding or GABA-dependent chloride uptake. These results indicate that carbamazepine administration after alprazolam discontinuation attenuates behavioral and neurochemical alterations associated with discontinuation.

Discontinuation of benzodiazepines after chronic use can produce a behavioral syndrome manifested by symptoms ranging from mild anxiety to seizures [1]. This syndrome has been reported to occur with particular frequency in patients taking alprazolam, especially in high doses for severe anxiety or panic disorder [2]. Several approaches have been suggested to prevent or limit discontinuation syndromes, including dose tapering and use of other psychotropic agents [see, for example, Refs. 3 and 4]. Several recent clinical studies reported the efficacy of the anticonvulsant carbamazepine in attenuating alprazolam discontinuation syndromes [5–8].

We have described previously a mouse model for benzodiazepine discontinuation characterized by motor hyperactivity and associated γ-aminobutyric acid$_A$ ($GABA_A$) receptor upregulation [9]. Discontinuation of alprazolam after 1 week of administration at 2 mg/kg/day in this system produces behavioral and receptor alterations 2–4 days after drug termination [10]. To evaluate the potential therapeutic value of carbamazepine in alprazolam discontinuation, we administered anticonvulsant doses of carbamazepine to mice for 1 week after discontinuation of alprazolam and assessed motor activity and $GABA_A$ receptor binding and function during this period.

* Corresponding author: Dr. Lawrence G. Miller, Box 1007, New England Medical Center, 750 Washington St., Boston, MA 02111. Tel. (617) 956-6878; FAX (617) 956-6409.

METHODS

Materials. Male CD1 mice (Charles River, Wilmington, MA), 6–8 weeks of age, were maintained on a 12-hr light/dark cycle and given food and water *ad lib*. Osmotic pumps were obtained from Alza (Palo Alto, CA). PEG400 was obtained from J. T. Baker (St. Louis, MO). [^{3}H]Flunitrazepam (sp. act. 70 Ci/mmol), [^{3}H]Ro15-1788 (sp. act. 81 Ci/mmol) and Solvable were purchased from New England Nuclear (Boston, MA), and [$^{36}Cl^-$] (sp. act. 25 Ci/g) was obtained from Amersham (Chicago, IL). Muscimol, carbamazepine and pentylenetetrazole were obtained from Sigma (St. Louis, MO). Alprazolam was a gift from Upjohn (Kalamazoo, MI). Flunitrazepam was a gift from Hoffman-La Roche (Nutley, NJ). Lorazepam was a gift from Wyeth (Radnor, PA). All other reagents were obtained from standard commercial sources.

Drug administration. Alprazolam, carbamazepine, and vehicle (PEG400) were administered by subcutaneously implanted osmotic pumps as previously reported [10]. The dose of alprazolam, 2 mg/kg/day, was chosen based on prior studies [10]. Carbamazepine doses were chosen based on prior studies in rodents [11]. To ensure that these doses were efficacious in mice, pentylenetetrazole-induced seizure determinations were performed as previously described [12]. Briefly, mice were treated with carbamazepine (25 or 100 mg/kg/day) or vehicle as described above. An acute (30 min) anticonvulsant dose of lorazepam, 10 mg/kg, was used as an anticonvulsant control. After 4 days, mice were

injected with pentylenetetrazole, 10 mg/mL i.v., until the onset of a full tonic-clonic convulsion. The convulsant dose of pentylenetetrazole was determined. Both doses of carbamazepine significantly ($P < 0.05$) increased convulsant pentylenetetrazole doses compared to vehicle. The effects of carbamazepine in both cases were similar to an anticonvulsant dose of lorazepam.

The treatment groups evaluated were:

Vehicle 7 days/Carbamazepine 25 mg/kg/day for 7 days

Vehicle 7 days/Carbamzepine 100 mg/kg/day for 7 days

Alprazolam 2 mg/kg/day for 7 days/Vehicle 7 days

Alprazolam 2 mg/kg/day for 7 days/Carbamazepine 25 mg/kg/day for 7 days

Alprazolam 2 mg/kg/day for 7 days/Carbamazepine 100 mg/kg/day for 7 days.

Open-field activity. Behavioral activity was monitored using an Opto-Varimex (Columbus Instruments, Columbus, OH). Total distance travelled was recorded for a 5-min period between 9:00 a.m. and 12:00 noon [13]. Animals were not previously handled or habituated to the apparatus, and all mice were tested only once.

Benzodiazepine binding. Benzodiazepine binding *in vivo* was performed as previously described [13]. Briefly, mice were injected i.v. with 3 μCi [^{3}H]Ro15-1788. After 20 min, animals were killed and brains were rapidly removed and dissected on ice. After weighing, brain regions were dissolved in Solvable (40° for 24 hr) and then counted by scintillation spectrometry. For benzodiazepine binding *in vitro*, synaptosomal membranes from mouse cerebral cortex were prepared as previously described [13]. Benzodiazepine binding was performed as previously described [13]. Briefly, to duplicate or triplicate samples was added [^{3}H]flunitrazepam, 0.1 to 20 nM. To an identical set of samples was added flunitrazepam, 10^{-5} M. After incubation at 4° for 45 min, samples were filtered using a Brandel M48R apparatus (Gaithersburg, MD) onto Whatman GF/B filters. Filters were washed twice with cold buffer and counted by scintillation spectrometry. In some experiments, cortices were hemisected and one section was used for benzodiazepine binding while the other section was used for carbamazepine concentrations.

GABA receptor function. $GABA_A$ receptor function in cortical synaptoneurosomes was determined as previously described [13, 14]. Briefly, cortical synaptosomes were prepared and resuspended in assay buffer (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, pH 7.4). After incubation for 10 min at 30°, to 100 μL of membranes was added 100 μL of a solution containing muscimol (1–50 μM) and [$^{36}Cl^-$], 0.2 μCi/mL assay buffer. After 6 sec the incubation was terminated by the addition of ice-cold assay buffer containing 6 μM picrotoxin and filtration on Whatman GF/C filters using a Brandel M24 apparatus. Filters were washed twice with cold buffer and counted by scintillation spectrometry.

Carbamazepine concentrations. Cortical tissue was weighed and homogenized in distilled water with a Polytron (Brinkmann, Lucerne; setting 7, 5 sec). Carbamazepine concentrations were determined

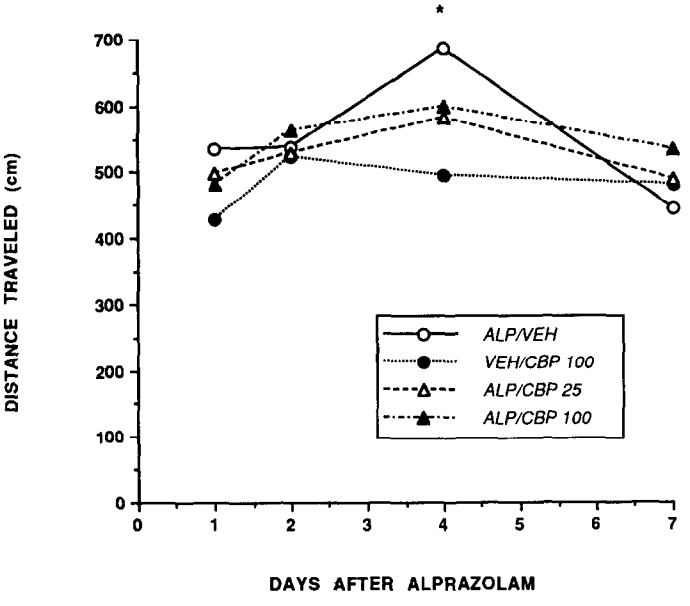

Fig. 1. Effects of alprazolam discontinuation on open-field activity. Activity was determined over a 5-min interval between 9:00 a.m. and 12:00 noon using a Digiscan apparatus. Results are means, N = 7–11 for each group. SEM were less than 20% of means. ALP/VEH = alprazolam for 7 days followed by vehicle for 7 days; VEH/CBP = vehicle for 7 days followed by carbamazepine for 7 days; ALP/CBP = alprazolam for 7 days followed by carbamazepine for 7 days; 100 = 100 mg/kg/day; 25 = 25 mg/kg/day. Key: (*) $P < 0.05$ vs control.

by high-performance liquid chromatography as previously described [15].

Data analysis. Binding data were analyzed using the EBDA programs [16]. Data were compared using analysis of variance with Dunnett's test or Student's *t*-test.

RESULTS

Open-field activity. As previously reported [10], open-field activity was increased in mice 4 days after alprazolam discontinuation when the mice were treated with vehicle alone during the discontinuation period (Fig. 1). In mice receiving carbamazepine, 25 or 100 mg/kg/day after alprazolam discontinuation, activity tended to increase at 4 days after alprazolam but this change did not achieve significance ($P < 0.15$ at both doses). Carbamazepine alone (100 mg/kg/day), administered after 1 week of vehicle, did not alter significantly open-field activity at 1, 2, 4 or 7 days of administration.

Benzodiazepine receptor binding. Benzodiazepine receptor binding *in vivo* was increased in cortex at 2 and 4 days and hypothalamus at 4 days after alprazolam discontinuation (Fig. 2), as previously reported [10]. Alterations in binding in hippocampus did not achieve significance. In mice treated with carbamazepine, 100 mg/kg/day, after alprazolam discontinuation, no significant change in binding was observed in cortex over the 7-day interval, and a nonsignificant trend towards increased binding occurred in hypothalamus at 4 days ($P < 0.10$). In mice treated with carbamazepine 25 mg/kg/day, (data not shown), binding was increased in cortex at day 2 but not day 4 compared to days 1 and 7, and was unchanged in hypothalamus. Neither dose of carbamazepine affected binding in hippocampus. Carbamazepine alone (100 mg/kg/day) administered for 1 week after vehicle did not affect benzodiazepine binding in any brain region evaluated. No treatment altered binding in cerebellum or pons-medulla in the period after alprazolam discontinuation.

Benzodiazepine receptor density *in vitro* in cortex was also increased at 4 days after alprazolam discontinuation. Treatment with carbamazepine at 25 (data not shown) or 100 mg/kg/day was associated with a nonsignificant increase in receptor density at 4 days ($P < 0.15$; Table 1). Apparent affinity was not affected by alprazolam or carbamazepine administration. Carbamazepine (100 mg/kg/day) after vehicle treatment did not affect benzodiazepine binding in cortex *in vitro*. For both alprazolam and carbamazepine, binding *in vivo* and *in vitro* at day 1 after discontinuation was similar to vehicle-treated controls (data not shown).

GABA-dependent chloride uptake. GABA-dependent chloride uptake was increased 4 days after alprazolam discontinuation compared to 1 day after discontinuation, as previously reported [10] (Fig. 3). Treatment with carbamazepine (100 mg/kg/day) after alprazolam attenuated this change, although the reduction in maximal chloride uptake did not achieve significance ($P < 0.10$). Uptake was similar at 1 and 4 days of carbamazepine (100 mg/kg/day) after vehicle administration.

Carbamazepine concentrations. Carbamazepine concentrations in cortex are presented in Table 2. In mice treated with carbamazepine after alprazolam administration, carbamazepine concentrations in cortex were not significantly different at 1, 2, 4 and 7 days of administration.

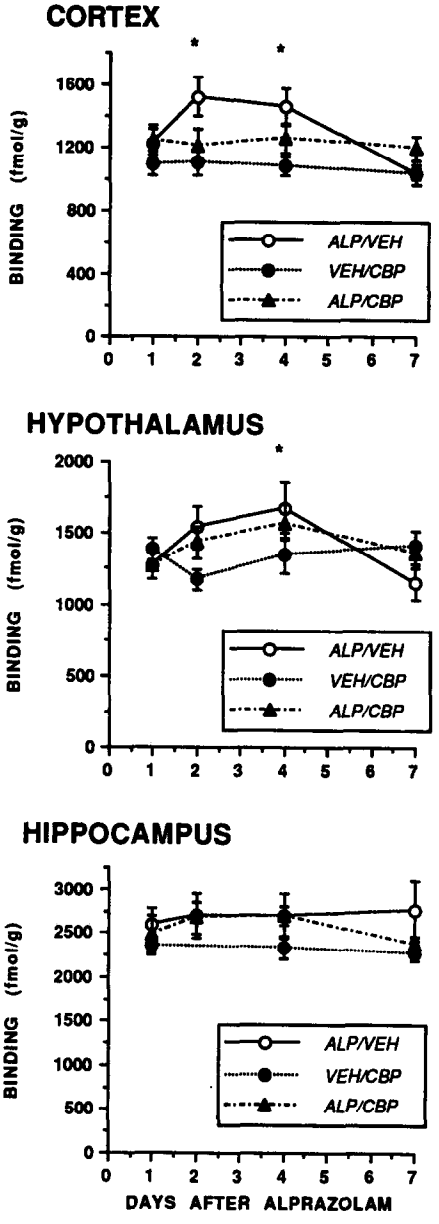

Fig. 2. Effects of alprazolam discontinuation on benzodiazepine binding *in vivo*. Binding was determined by uptake of [^{3}H]Ro15-1788. Results are means ± SEM, N = 6–10 for each group. ALP/VEH = alprazolam for 7 days followed by vehicle for 7 days; VEH/CBP = vehicle for 7 days followed by carbamazepine, 100 mg/kg/day, for 7 days; ALP/CBP = alprazolam for 7 days followed by carbamazepine for 7 days. Key: (*) $P < 0.05$ for ALP/VEH at days 2 or 4 vs day 1.

DISCUSSION

As previously described, alprazolam administration for 1 week in mice produced a discontinuation

Table 1. Effects of alprazolam discontinuation on benzodiazepine binding in cortex *in vitro*

Treatment/Day	K_d (mM)	B_{max} (pmol/mg protein)
Alprazolam/Vehicle		
1	1.14 ± 0.14	1.28 ± 0.08
2	0.91 ± 0.04	1.42 ± 0.09
4	1.06 ± 0.07	1.76 ± 0.27*
Vehicle/Carbamazepine		
2	1.08 ± 0.17	1.20 ± 0.06
4	1.17 ± 0.13	1.25 ± 0.10
7	0.98 ± 0.15	1.29 ± 0.06
Alprazolam/Carbamazepine		
1	0.90 ± 0.06	1.23 ± 0.12
2	0.92 ± 0.05	1.20 ± 0.13
4	0.98 ± 0.17	1.46 ± 0.09
7	1.06 ± 0.11	1.23 ± 0.11

Benzodiazepine binding was performed in cortical membranes using [^{3}H]flunitrazepam. Mice were treated with alprazolam, 2 mg/kg/day, or vehicle for 1 week followed by carbamazepine, 100 mg/kg/day or vehicle for 1 week. Results are means ± SEM, N = 3–4 determinations from 1–2 membrane preparations. Day = days after alprazolam discontinuation.

* $P < 0.05$ vs day 1.

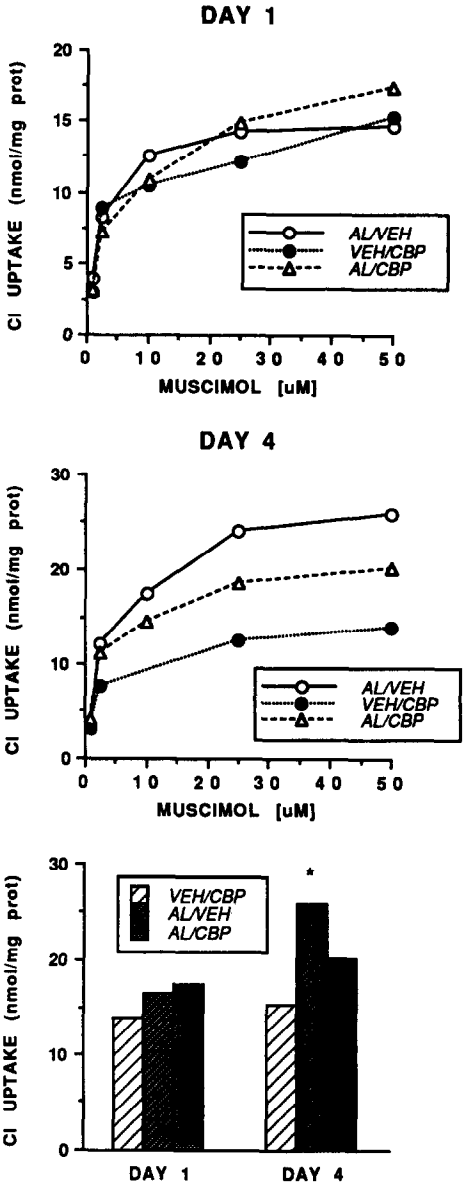

Fig. 3. Effects of alprazolam discontinuation on GABA-dependent chloride uptake in cortex. Uptake was determined in cortical synaptoneurosomes over a 6-sec interval. Results are means, N = 3–5 for each group. SEM were less than 20% of means. AL/VEH = alprazolam for 7 days followed by vehicle for 7 days; VEH/CBP = vehicle for 7 days followed by carbamazepine for 7 days; AL/CBP = alprazolam for 7 days followed by carbamazepine for 7 days. As indicated in the bottom panel, uptake at 50 μM muscimol was significantly greater (* = $P < 0.05$) for AL/VEH at day 4 compared to day 1, and for AL/VEH compared to VEH/CBP at day 4. Uptake at 50 μM muscimol was not significantly different at day 4 for AL/VEH and AL/CBP ($P < 0.10$).

syndrome characterized by increased open-field activity and $GABA_A$ receptor upregulation at 2–4 days after drug discontinuation [10]. These alterations were attenuated by carbamazepine administration after alprazolam discontinuation at two anticonvulsant doses of carbamazepine. Carbamazepine alone at either dose did not affect open-field activity or $GABA_A$ receptor parameters. Thus, these data appear to support the potential efficacy of carbamazepine in reducing the effects of alprazolam discontinuation.

It should be pointed out that carbamazepine did not completely reverse the changes in open-field activity or in receptor binding and function associated with alprazolam discontinuation. In addition, although both doses of carbamazepine were anticonvulsant, the higher dose appeared to be more effective in returning activity and binding values toward baseline. Thus, carbamazepine appears to be partially efficacious in attenuating alprazolam discontinuation effects, with a probable dose relationship. The greater variance in carbamazepine-treated compared to vehicle-treated mice might also indicate greater individual variability in responses to carbamazepine.

The possible mechanisms whereby carbamazepine might alter alprazolam discontinuation effects remain uncertain. Both electrophysiological and ligand binding evidence argue against a direct effect of carbamazepine on post-synaptic $GABA_A$ receptors [11, 17]. Limited evidence indicates that carbamazepine can increase synaptosomal GABA concentrations, perhaps by decreasing turnover [18]. If such an effect occurred during alprazolam discontinuation, carbamazepine might be expected to augment benzodiazepine binding and GABA-dependent chloride uptake rather than to decrease these parameters. Some evidence also supports effects of carbamazepine at the peripheral benzodiazepine receptor located on non-neuronal cells, although the involvement of this site in discontinuation effects remains speculative [19].

Table 2. Carbamazepine concentrations in cortex

Day	Carbamazepine (μg/g)
1	5.76 ± 0.98
2	4.92 ± 0.62
4	5.19 ± 1.34
7	4.40 ± 1.11

Mice were treated with alprazolam, 2 mg/kg/day for 7 days followed by carbamazepine, 100 mg/kg/day, for 7 days. Carbamazepine concentrations in cortex were determined by high-performance liquid chromatography. Results are means ± SEM, N = 3 for 1, 4 and 7 days, N = 4 for 2 days. There were no significant differences. Day = days of carbamazepine administration.

It is possible that carbamazepine exerts its effects through inhibition of sodium channel-mediated neurotransmitter release [11]. If this is the case, carbamazepine may act in benzodiazepine discontinuation by suppressing neurotransmitter release by excitatory neurons in the presence of reduced GABA-mediated inhibition. There is also evidence that carbamazepine enhances catecholamine and monoamine function, which in turn may effect behavior and neurochemcial parameters during drug discontinuation [11]. Other hypotheses include an effect of carbamazepine on a putative endogenous benzodiazepine ligand, or an indirect effect via $GABA_B$ receptor alterations associated with carbamazepine [20], or alterations in $GABA_A$ receptor subunit gene expression. Changes in gene expression have been reported during chronic benzodiazepine administration [21] but no data are available related to discontinuation.

Our results in a mouse model parallel recent results in clinical studies, which indicate a partial reduction in the severity of alprazolam discontinuation symptoms associated with carbamazepine administration [7, 8]. Variation among individuals in clinical studies may indicate that specific individuals are more likely to respond to carbamazepine; similar variability occurred in animal studies. Results in this animal model also indicate that the reduction in behavioural discontinuation effects is accompanied by reductions in neurochemical alterations, specifically benzodiazepine binding and $GABA_A$ receptor function. The effects of carbamazepine on both behavioral and neurochemical parameters support the association between behavioral and neurochemical parameters previously described in benzodiazepine discontinuation [9, 10]. The mouse model used in this study appears to be appropriate for additional studies of carbamazepine attenuation of benzodiazepine discontinuation, and for studies of other methods to limit discontinuation effects.

Acknowledgements—The authors thank Young Shim for assistance. This work was supported in part by Grants DA-05258, MH-34223 and AG-01006 from the U.S. Public Health Service and by a Grant-in-Aid from The Upjohn Company. Dr. Miller is the recipient of a Faculty Development Award in Clinical Pharmacology from the Pharmaceutical Manufacturers Association Foundation.

REFERENCES

1. Rickels K, Case WG, Schweizer E, Garcia-Espana F and Fridman R, Benzodiazepine dependence: Management of discontinuation. *Psychopharmacol Bull* **26**: 63–68, 1990.
2. Roy-Byrne PP and Hommer D, Benzodiazepine withdrawal: Overview and implications for the treatment of anxiety. *Am J Med* **84**: 1041–1052, 1988.
3. Schweizer E and Rickels K, Failure of buspirone to manage benzodiazepine withdrawal. *Am J Psychiatry* **143**: 1590–1592, 1986.
4. Schweizer E, Rickels K, Case WG and Greenblatt DJ, Long-term therapeutic use of benzodiazepines. II. Effects of gradual taper. *Arch Gen Psychiatry* **47**: 908–915, 1990.
5. Klein E, Uhde TW and Post RM, Preliminary evidence for the utility of carbamazepine in alprazolam withdrawal. *Am J Psychiatry* **143**: 235–236, 1986.
6. Ries RK, Roy-Byrne PP, Ward NG, Neppe V and Cullison S, Carbamazepine treatment for benzodiazepine withdrawal. *Am J Psychiatry* **146**: 536–537, 1989.
7. Rickels K, Schweizer E, Case WG and Greenblatt DJ, Long-term therapeutic use of benzodiazepines. I. Effects of abrupt discontinuation. *Arch Gen Psychiatry* **47**: 899–907, 1990.
8. Schweizer E, Rickels K, Case WG and Greenblatt DJ, Carbamazepine treatment in patients discontinuing long-term benzodiazepine therapy. *Arch Gen Psychiatry* **48**: 448–452, 1991.
9. Miller LG, Greenblatt DJ, Summer WR and Shader RI, Chronic benzodiazepine administration: II. Discontinuation syndrome is associated with upregulation of γ-aminobutyric acid$_A$ receptor complex binding and function. *J Pharmacol Exp Ther* **246**: 177–182, 1988.
10. Lopez F, Miller LF, Greenblatt DJ, Chesley S, Schatzki A and Shader RI, Chronic administration of benzodiazepines: V. Rapid onset of behavioral and neurochemical alterations after discontinuation of alprazolam. *Neuropharmacology* **29**: 237–241, 1990.
11. MacDonald RL, Antiepileptic drug actions. *Epilepsia* (Suppl 1): S19–S28, discussion S64–S68, 1988.
12. Schatzki A, Lopez F, Greenblatt DJ, Shader RI and Miller LG, Lorazepam discontinuation promotes "inverse agonist" effects of benzodiazepines. *Br J Pharmacol* **98**: 451–454, 1989.
13. Miller LG, Greenblatt DJ, Barnhill JG and Shader RI, Chronic benzodiazepine administration: I. Tolerance is associated with benzodiazepine receptor down-regulation and decreased γ-aminobutyric acid$_A$ receptor function. *J Pharmacol Exp Ther* **246**: 170–176, 1988.
14. Miller LG, Chronic benzodiazepine administration: From the patient to the gene. *J Clin Pharmacol* **31**: 492–495, 1991.
15. Szabo GK, Pylilo RJ, Perchalski RJ and Browne TR, Simultaneous separation and determination (in serum) of phenytoin and carbamazepine and their deuterated analogues by high-performance liquid chromatography–ultraviolet detection for tracer studies. *J Chromatogr* **535**: 271–277, 1990.
16. McPherson GA, A practical computer based approach to the analysis of radioligand binding experiments. *Comput Programs Biomed* **17**; 107–114, 1983.
17. Pitkänen A, Saano V, Tuomisto L and Reikkinen PJ, Effect of anticonvulsant drugs on (^{35}S)*t*-butylbicyclophosphorothionate binding *in vitro* and *ex vivo*. *Pharmacol Toxicol* **61**: 103–106, 1987.
18. Post RM, Time course of clinical effects of carbamazepine: Implications for mechanisms of action. *J Clin Psychiatry* **49**(Suppl): 35–48, 1988.
19. Bender AS and Hertz L, Evidence for involvement

of the astrocytic benzodiazepine receptor in the mechanism of action of convulsant and anticonvulsant drugs. *Life Sci* **43**: 477–484, 1988.

20. Motohashi N, Ikawa K and Kariya T, $GABA_B$ receptors are up-regulated by chronic treatment with lithium or carbamazepine. GABA hypothesis of affective disorders? *Eur J Pharmacol* **166**: 95–99, 1989.
21. Kang I and Miller LG, Decreased $GABA_A$ receptor subunit mRNA concentrations following chronic lorazepam administration. *Br J Pharmacol* **103**: 1285–1287, 1991.