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Lorazepam discontinuation promotes 'inverse agonist' effects of benzodiazepines

Andrew Schatzki, Fred Lopez, David J. Greenblatt, Richard I. Shader & [1]Lawrence G. Miller

Division of Clinical Pharmacology, Departments of Psychiatry, Medicine and Pharmacology, Tufts University School of Medicine and New England Medical Center, Boston, MA, U.S.A.

1 The effects of lorazepam discontinuation on responses to benzodiazepine agonists and antagonists were studied in mice.

2 The convulsant dose of pentylenetetrazol was decreased after an acute dose of lorazepam (0.5 $mg\,kg^{-1}$) at 4 days after drug discontinuation, compared to 1 or 7 days after discontinuation or to vehicle treatment.

3 The percentage of mice undergoing convulsions after an acute dose of FG 7142 (40 $mg\,kg^{-1}$) was increased at 4 days after lorazapam discontinuation, compared to 1 or 7 days after discontinuation or to vehicle treatment.

4 After an acute dose (0.5 $mg\,kg^{-1}$), lorazepam concentrations in cortex tended to be greater in lorazepam-treated compared to vehicle-treated mice at 4 days after discontinuation compared to 1 and 7 days.

5 These data indicate a shift toward reduced agonist sensitivity and increased inverse agonist sensitivity in mice 4 days after lorazepam discontinuation.

Introduction

Discontinuation syndromes associated with benzodiazepine administration have been reported widely in man and may limit clinical use of benzodiazepines (Petursson & Lader, 1981; Owen & Tyrer, 1983; Woods *et al.*, 1987; 1988; Roy-Byrne & Hommer, 1988). However, the mechanism of benzodiazepine discontinuation syndromes remains uncertain. Studies in animal models have reproduced a behavioural discontinuation syndrome (File, 1982; Miller *et al.*, 1988c), in some cases indicating an increase in sensitivity to benzodiazepine 'inverse agonists' after discontinuation of flurazepam (Little *et al.*, 1987a,b; Nutt *et al.*, 1988). Indeed, one group suggested the presence of a 'withdrawal shift' in the direction of inverse agonist effects associated with benzodiazepine discontinuation (Little *et al.*, 1986).

Benzodiazepines exert their effects by binding at a specific site on the $GABA_A$ receptor complex located on postsynaptic neurones (Haefely *et al.*, 1985). Alterations in this site associated with chronic benzodiazepine use have been described by several groups. Although prior studies addressing receptor alterations after benzodiazepine discontinuation in animals produced conflicting results (Crawley *et al.*, 1982; Scharf & Feil, 1983), we have recently described a model of lorazepam discontinuation which produces behavioural changes consistent with prior studies and changes in binding and function at the $GABA_A$ receptor (Miller *et al.*, 1988c). The functional effects of benzodiazepines in this model, and the occurrence of a 'withdrawal shift', have not been evaluated.

To assess the efficacy of benzodiazepine agonists and inverse agonists in a model of benzodiazepine discontinuation, we evaluated the anticonvulsant response of an acute dose of lorazepam and the convulsant response of an acute dose of the inverse agonist FG-7142 in mice after discontinuation of chronic lorazepam.

Methods

Male CD-1 mice, 6–8 weeks of age, were obtained from Charles River laboratories (Wilmington, MA), housed under a 12 h light-dark cycle, and given laboratory chow and water *ad libitum*.

[1] Author for correspondence at: Division of Clinical Pharmacology, Box 1007, Tufts-New England Medical Center, 171 Harrison Avenue, Boston, MA 02111, U.S.A.

Lorazepam, 2 mg kg^{-1} daily, was administered chronically as previously described (Miller *et al.*, 1988b). Briefly, lorazepam was dissolved in PEG 400 and placed in Alzet 2001 or 2002 osmotic pumps. Pumps were implanted subsutaneously under brief ether anaesthesia. Control mice received pumps containing vehicle alone. Pumps were removed after 7 days. To ensure that behavioural changes occurred as previously described, open-field activity was performed in groups of lorazepam- and vehicle-treated mice 4 days after drug discontinuation. Open-field activity was greater in lorazepam-treated compared to vehicle-treated mice at this time (LRZ, 1353 ± 61 cm, $n = 9$; Vehicle, 1160 ± 83 cm, $n = 7$; mean ± s.e. mean, $P < 0.05$), consistent with prior results demonstrating increased motor activity 4 days after discontinuation of chronic lorazepam infusion (Miller *et al.*, 1988c).

For determination of benzodiazepine agonist efficacy, mice were injected i.p. with a single dose of lorazepam, 0.5 mg kg^{-1}. After 20 min, animals were injected i.v. via the tail vein with a continuous infusion of pentylenetetrazol, 10 mg kg^{-1} at a rate of 0.6 ml min^{-1} (Nutt *et al.*, 1986). The infusion was terminated at the onset of a full tonic-clonic seizure, and the amount required for seizure induction was recorded. Experimental groups were analyzed at 1, 4 and 7 days after the discontinuation of chronic treatment.

To control for possible differences in brain uptake of lorazepam, groups of chronically lorazapam- and vehicle-treated mice were injected with an acute dose of lorazepam as above. After 20 min, animals were killed and brains rapidly removed and dissected on ice. Cortices were homogenized in 1 ml water with a Polytron (setting 7, 10 s) and frozen at −20°C for subsequent lorazepam determinations. Lorazepam concentrations in tissue were determined by gas-liquid chromatography as previously described, with oxazepam used as the internal standard (Greenblatt *et al.*, 1978).

To determine benzodiazepine inverse agonist efficacy, chronically treated mice were injected with a single dose of FG-7142, 40 mg kg^{-1} i.p. (Little *et al.*, 1984). The occurrence of a full tonic-clonic seizure was recorded. Groups were similar to those evaluated for benzodiazepine agonist efficacy.

Data were analyzed by analysis of variance with Dunnett's correction for continuous data, or Chi-Square for discrete data, or the Mann-Whitney test for non-parametric data.

Materials

Osmotic pumps (models 2001, 2002) were obtained from Alza (Palo Alto, CA). FG-7142 (n-methyl-β-carboline-3-carboxamide) was obtained from Research Biochemicals (Natick, MA). Pentylenetetrazol was obtained from Sigma (St. Louis, MO). Lorezepam was a generous gift from Wyeth Laboratories (Radnor, PA). PEG-400 was obtained from J.T. Baker (St. Louis, MO). All other reagents were obtained from standard commercial sources.

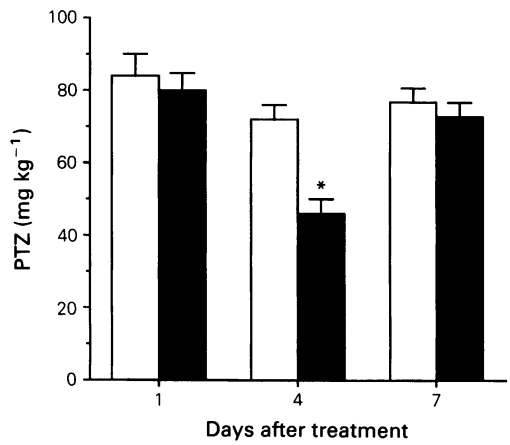

Figure 1 Effects of lorazepam discontinuation on anticonvulsant effects of acute lorazepam. Mice were treated with vehicle or lorazepam as described in the text. Mice then received lorazepam, 0.5 mg kg^{-1}, and 20 min later an infusion of pentylenetetrazol(PTZ), 10 mg ml^{-1} at 0.6 ml min^{-1}. Infusion was terminated at the onset of a full tonic-clonic seizure. Open columns represent vehicle, solid columns represent lorazepam treatment. Results are mean, $n = 15$ for each group; s.e. mean shown by vertical bars. Asterisk denotes $P < 0.05$ vs. vehicle and lorazepam at days 1 and 7.

Results

At 1, 4, and 7 days after drug discontinuation, mice treated with vehicle had similar seizure thresholds for pentylenetetrazol after a single dose of lorazepam (Figure 1). Results in lorazepam-treated mice 1 and 7 days after drug discontinuation produced similar results; similar doses of pentylenetetrazol were required to induce seizures at these time points, and results were similar to those in vehicle-treated mice. However, 4 days after lorazepam discontinuation, significantly less pentylenetetrazol was required for seizure induction, indicating a decreased anticonvulsant effect of lorazepam in these mice. Results at 4 days were significantly different from lorazepam-treated mice 1 and 7 days after drug discontinuation, and from all vehicle-treated groups.

Four days after drug discontinuation, lorazepam concentrations after an acute dose in lorazepam-treated mice tended to be greater than concentra-

tions in vehicle-treated mice (vehicle, $206 \pm 34\,ng\,g^{-1}$; chronic lorazepam $314 \pm 19\,ng\,g^{-1}$; mean $\pm$ s.e.mean, $n = 5$ in each group, $P = 0.06$ Mann-Whitney). Thus, brain uptake of benzodiazepines may be altered in mice after benzodiazepine discontinuation. However, the finding of possibly increased lorazepam concentrations in brain concurrent with decreased anticonvulsant effect further emphasizes the decreased efficiacy of lorazepam in these experiments.

In groups of mice at 1, 4, and 7 days after vehicle discontinuation, administration of the inverse benzodiazepine agonist FG 7142 caused tonic-clonic seizures in similar numbers of mice (Day 1: 20%; Day 4: 21%; Day 7: 25%; Figure 2). Similar results were observed in mice 1 (18.5%) and 7 (27%) days after discontinuation of chronic lorazepam. However, at 4 days after lorazepam discontinuation, a significantly greater percentage of animals (58%) sustained tonic-clonic seizures compared to days 1 and 7 post-lorazepam, and compared to all vehicle-treated groups. Although the group size at 7 days was less than 1 and 4 days (7 days $n = 12$, 1 and 4 days $n = 15$–18), the results are significant ($P < 0.05$) in the lorazepam group.

Discussion

A discontinuation syndrome associated with benzodiazepine use has been described in man and in several animal models (Petursson & Lader, 1981; File, 1982; Owen & Tyrer, 1983; Roy-Byrne & Hommer, 1988; Woods *et al.*, 1987; 1988; Miller *et al.*, 1988c). In animals, this syndrome is characterized by increased motor activity (Miller *et al.*, 1988c), and increased sensitivity to the effects of inverse agonist

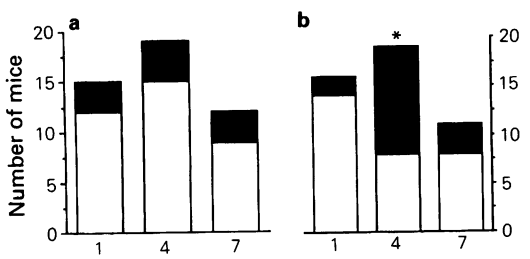

Figure 2 Effects or lorazepam discontinuation on convulsant responses to FG 7142. Mice were treated with vehicle (a) or lorazepam (b) as described in the text. Mice then received FG 7142, $40\,mg\,kg^{-1}$, and were observed for the development of a full tonic-clonic seizure. Solid columns represent number of mice sustaining seizures; open columns represent mice without seizures. Asterisk denotes $P < 0.05$ vs. vehicle and lorazepam at days 1 and 7.

compounds (Little *et al.*, 1987a,b). Indeed, Little and colleagues coined the term 'withdrawal shift' to describe the increased hypothermic and convulsant effects of inverse agonists after flurazepam discontinuation in mice (Little *et al.*, 1986). We have used the term 'discontinuation syndrome' rather than 'withdrawal syndrome' to avoid connotations associated with opiate withdrawal (Miller, 1988). Discontinuation syndromes have not been observed in all systems or for some behavioural effects (Nutt *et al.*, 1988). This may be due in part to use of various benzodiazepines and to choice of time points for testing. In general, benzodiazepine discontinuation syndromes appear to occur at increasing intervals after the last drug dose as drug half-life increases (Greenblatt & Shader, 1978). Symptoms may not be present until days after discontinuation in animals treated with agents with prolonged half-lives or persistent metabolites, such as diazepam or flurazepam (Greenblatt *et al.*, 1983). Thus, assessment of symptoms soon after discontinuation of these agents may fail to detect behavioural or neurochemical alterations. For example, few alterations in seizure susceptibility were observed in mice 24 and 48 h after discontinuation of chronic flurazepam (Nutt *et al.*, 1988). However, the metabolite *n*-desalkyl flurazepam has high receptor occupancy (low K_i) and a prolonged half-life, so that symptons may not occur until days after drug discontinuation (Miller *et al.*, 1988a). It is possible that substantial concentrations of the metabolites were present in brain at 24 h and perhaps 48 h after drug termination.

Our results indicate that 4 days after a course of lorazepam previously demonstrated to induce behavioural tolerance, mice exhibited a decrease in the anticonvulsant effect of acute lorazepam to a pentylenetetrazol stimulus. To exclude a pharmacokinetic explanation for this effect, concentrations of lorazepam were determined in brain after the acute dose. Cortical lorazepam concentrations tended to be increased in lorazepam-treated mice, making it likely that brain uptake of lorazepam was increased at this time point. These data emphasize the decrease in efficacy of lorazepam after drug discontinuation, since increased concentrations would be expected to enhance anticonvulsant effects. Our results are consistent with a shift toward reduced agonist sensitivity as suggested by Little *et al.* (1986).

We also observed an increase in the number of mice exhibiting seizures after a fixed dose of the inverse agonist FG 7142 in mice 4 days after lorazepam discontinuation. These results are consistent with those obtained using pentylenetetrazol, also indicating enhanced efficacy of inverse agonist compounds at this time point. Alterations in brain uptake may contribute to the differential effects of FG 7142. Since acute lorazepam concentrations

tended to be increased in mice after chronic lorazepam discontinuation, it is possible that brain uptake of FG-7142 is also increased. Such an alteration could contribute to the increase in convulsant effect observed in lorazepam compared to vehicle-treated mice.

These data indicate that several indices of $GABA_A$ receptor function are altered after lorazepam discontinuation. Behavioural effects at 4 days post-lorazepam include altered open-field activity (Miller *et al.*, 1988c) and benzodiazepine efficacy. As previously reported, this time point is associated with increased benzodiazepine- and GABA-related chloride uptake (Miller *et al.*, 1988c). The mechanism for increased inverse agonist effects remains uncertain. It is possible that the observed increases in receptor number or availability might also be associated with altered coupling between benzodiazepine and GABA binding, so that an inverse agonist effect predominates. Alternatively, alterations in putative endogeneous ligands might result in an imbalance in favour of inverse agonist compounds (Medina *et al.*, 1989). The simultaneous occurrence of behavioural and neurochemical alterations after lorazepam discontinuation indicates that this system may be valuable as a model for benzodiazepine discontinuation.

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