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The new antiepileptic drug levetiracetam normalises chlordiazepoxide withdrawal-induced anxiety in mice

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Abstract

Some antiepileptic drugs have been used with success to counteract withdrawal symptoms following chronic use of sedatives, hypnotics or alcohol. We evaluated the potential of levetiracetam (Keppra™), a new antiepileptic drug, to prevent benzodiazepine withdrawal in an animal model sensitive to the anxiogenic effect resulting from drug cessation. The effects of levetiracetam (17 and 54 mg/kg) given intraperitoneally (i.p.) were determined on anxiety induced in female NMRI mice by withdrawal from 21 days of chronic administration of chlordiazepoxide. Administration of chlordiazepoxide was i.p. twice daily, in increments of 2 mg/kg, from 10 up to 40 mg/kg. Anxiety was evaluated using an elevated plus-maze test 24-h after chlordiazepoxide withdrawal. Discontinuation of chronic chlordiazepoxide induced a significant anxiogenic profile in the plus-maze test mainly characterised by a decrease in open arm exploration. This effect was dose-dependently prevented by administration of levetiracetam during the withdrawal period. The highest dose tested (54 mg/kg) induced statistically significant effects on all variables recorded but had no effect upon plus-maze exploration in normal mice. This suggests that the observed effects are dependent upon the level of stress or anxiety of the animals. These results support potential efficacy of levetiracetam in the benzodiazepine withdrawal syndrome.

Keywords: Antiepileptic drug; Anxiety; Benzodiazepine; Keppra™; Levetiracetam; Withdrawal

1. Introduction

Benzodiazepines are known to induce tolerance and dependence in man (Petursson, 1994; Woods et al., 1987). Abrupt withdrawal from prolonged treatment with benzodiazepines can lead to an abstinence syndrome with symptoms depending upon the duration of the treatment and the daily dosage (Woods et al., 1987). Symptoms include increased anxiety, irritability, sleep disorders, tremors, weight loss, etc., of which increased anxiety predominates (Rickels et al., 1990; Petursson, 1994). It is possible to produce analogous symptoms in animals, at least in part (Emmett-Oglesby et al., 1990). It has been shown, for example, that withdrawal from chronic treatment with chlordiazepoxide or diazepam results in weight loss, anorexia (Leathley and Goudie, 1992) and in an "anxiogenic" state in rats, detectable in the elevated plus-maze test (File and Andrews, 1991).

Various compounds, with or without action on the GABAergic (GABA) receptor complex, have been reported to ameliorate specific behavioural consequences resulting from benzodiazepine withdrawal in animal models (File and Andrews, 1993; Tsuda et al., 1998; Reddy and Kulkarni, 1997; Goudie and Leathley, 1995; Saad et al., 1997). In particular, certain Ca^{2+} channel blockers have been shown to be of value in ameliorating benzodiazepine withdrawal symptoms in mice (Chugh et al., 1992; Saad et al., 1997). In man, pharmacological treatments of benzodiazepine withdrawal have used, with various success, different types of drugs like antidepressants, beta-blockers, serotonergic anxiolytics or antiepileptic drugs to treat specific symptoms concomitant to withdrawal (Rickels et al., 1999). Sodium valproate and carbamazepine are two antiepileptic drugs that have been proposed as adjunctive therapy during withdrawal from sedative–hypnotics or alcohol (Pages and Ries, 1998). Both drugs have been proposed to 'normalise' the $GABA_A$ receptor function altered with chronic exposure to sedative–hypnotic drugs (Harris et al., 2000). However, the exact pathophysiological basis of benzodiazepine withdrawal is not clearly understood and both sodium valproate and carbamazepine have multiple actions in the central nervous system

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Table1
Dosing schedules for mice receiving chronic administration of chlordiazepoxide (CDP), with or without withdrawal and replacement treatment with levetiracetam (LEV)

Group ($n=17$)	Day 1–Day 21 (i.p. 2 × daily)	Day 22 (i.p. 2 × daily)	Day 23 (i.p. 60 min pre-test)
Control (SAL–SAL)	saline	saline	saline
Chlordiazepoxide non-withdrawal (CDP–CDP)	CDP 10–40 mg/kg	CDP 40 mg/kg	CDP 40 mg/kg
Chlordiazepoxide withdrawal (CDP–SAL)	CDP 10–40 mg/kg	saline	saline
Chlordiazepoxide withdrawal + levetiracetam 17 mg/kg (CDP–LEV 17 mg/kg)	CDP 10–40 mg/kg	LEV 17 mg/kg	LEV 17 mg/kg
Chlordiazepoxide withdrawal + levetiracetam 54 mg/kg (CDP–LEV 54 mg/kg)	CDP 10–40 mg/kg	LEV 54 mg/kg	LEV 54 mg/kg

(Moshé, 2000) but share a common action relating to blockade of certain subtypes of Ca^{2+} channels (Moshé, 2000).

Levetiracetam (Keppra™) is a pyrrolidine derivative, recently registered as add-on treatment of refractory partial onset seizures in adults. In animals, levetiracetam selectively suppress seizures in various rodent models of chronic epilepsy (Gower et al., 1992; Klitgaard et al., 1998). Its mechanism of action does not appear to involve the main cellular mechanisms associated with classical antiepileptic drugs (Sills et al., 1997; Zona et al., 2001). Thus, levetiracetam appears devoid of conventional GABAergic effects and reveals no impact on voltage-activated Na^{+} and T-type (low-voltage) Ca^{2+} channels in cultured neurons (Zona et al., 2001). However, levetiracetam reduces bicuculline-induced hippocampal hyperexcitability both in vivo (Margineanu and Wulfert, 1997) and in vitro (Birnstiel et al., 1997). This effect appeared calcium-ion related (Wulfert and Margineanu, 1998) and corroborates recent findings showing that levetiracetam reduces high voltage-activated Ca^{2+} currents in rat hippocampus (Niespodziany et al., 2001). Furthermore, it was also recently observed that levetiracetam opposes the inhibition by negative allosteric modulators of GABA- and glycine-gated currents (Rigo et al., 2000). Thus, the multiple mechanisms of levetiracetam make it an interesting drug candidate for the treatment of benzodiazepine withdrawal-induced central nervous system hyperexcitability (Ladewig, 1984; Davies et al., 1988).

The aim of the present study was to evaluate the ability of levetiracetam to prevent benzodiazepine withdrawal in an animal model sensitive to the anxiogenic effect resulting from such a withdrawal. We studied the effect of levetiracetam against chlordiazepoxide withdrawal-induced anxiety in mice, measured in the elevated plus-maze test.

The protocol described below complies with the European Community guidelines for the use of experimental animals and was approved by the local ethics committee for laboratory animals according to Belgian law.

2. Methods and materials

2.1. Animals

Female NMRI mice (UCB), aged 5 weeks and weighing between 22 and 27 g at the beginning of the experiments, were used. The mice were housed in groups in transparent plastic cages containing a bedding layer of sawdust under a 12-h light–dark cycle with lights on at 0700 h. The mice were allowed ad lib access to food and water.

2.2. Drugs and solutions

Levetiracetam (UCB Pharma) was dissolved in 0.9% saline solution. Chlordiazepoxide hydrochloride (RBI) was also dissolved in 0.9% saline solution. A dose volume of 0.1-ml/10 g body weight was used. Control groups received an equivalent dose-volume of 0.9% saline solution. All drugs were injected intraperitoneally (i.p.). The doses of levetiracetam chosen were the doses reported to be active in various animal models of epilepsy (Gower et al., 1992; Klitgaard et al., 1998).

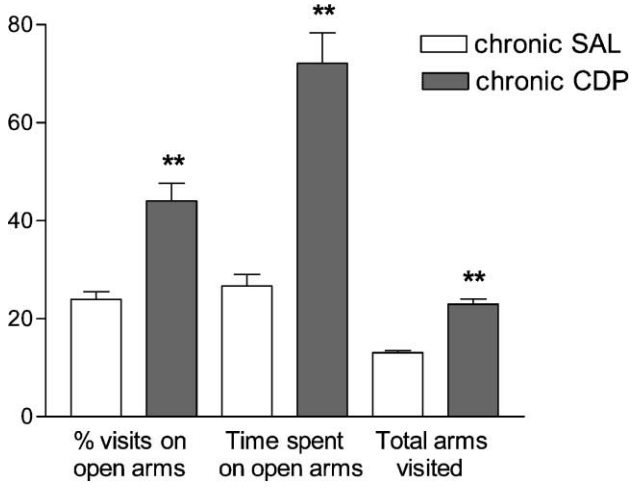

Fig. 1. Effect of repeated administration of chlordiazepoxide on behaviour of mice tested in the elevated plus-maze. Mice were chronically injected with chlordiazepoxide (chronic CDP) or saline (chronic SAL) for 23 days progressing in daily increments of 2 mg/kg, from 10 up to 40 mg/kg twice daily. Mice were evaluated in the elevated plus-maze 60 min after the last injection. Results are expressed as mean ± S.E.M. ** $P<0.001$ (Student's t-test).

2.3. Apparatus

The elevated plus-maze was based on the description by Lister (1987) and consisted of two open arms (29-cm long × 5-cm wide) and two closed arms (29 × 5 cm with 15-cm-high walls) forming a square cross with a 5-cm square centre piece. The floor of the arms and the centre-piece were made of grey plastic, and the walls of the closed arms were made of transparent plastic. The apparatus was mounted 40 cm above the floor.

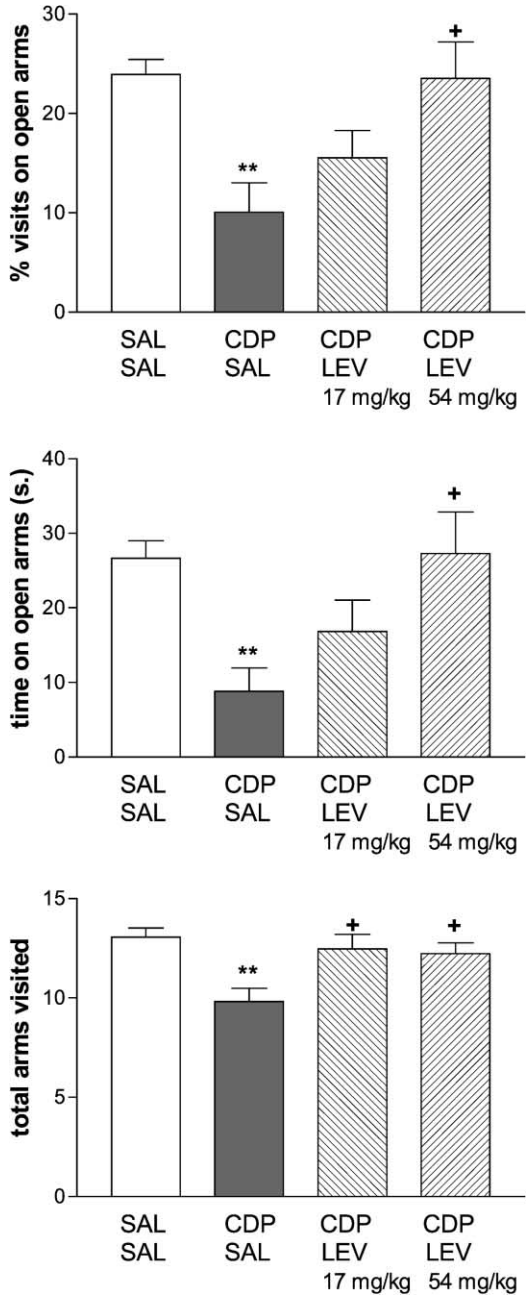

Fig. 2. Effect of levetiracetam (LEV) 17 and 54 mg/kg on chlordiazepoxide withdrawal-induced anxiety in mice evaluated in the elevated plus maze test. Mice were chronically injected with chlordiazepoxide (CDP) or saline (SAL) for 21 days, twice daily. On day 22, the injections of chlordiazepoxide were replaced with saline (CDP–SAL) or levetiracetam (CDP–LEV). Control animals received the same amount of saline injections during the withdrawal period (SAL–SAL). Mice were evaluated on day 23, 60 min after treatment. Results are expressed as mean ± S.E.M. ** $P<0.001$ (Student's t-test) compared to SAL–SAL group. + $P<0.05$ (Dunnett's test) compared to CDP–SAL group.

Table 2
Effect of levetiracetam given acutely 60 min before plus-maze testing (day 23) to mice having received saline twice daily during 22 days

Group ($N=9$)	% Visits on open arms	Time spent on open arms (s)	Total number of arms visited
Control	24 (2.4)	22 (3.0)	12 (0.5)
Levetiracetam (54 mg/kg)	26 (2.9)	31 (4.3)	15 (1.0)

Results are means with S.E.M. in parentheses. No significant difference between group was found.

2.4. Procedure

Groups of eight mice were injected twice daily (once between 0900 and 1000 h and again between 1500 and 1600 h) with chlordiazepoxide or saline for 21 days. Dosing started with 10 mg/kg on day 1 and was increased in steps of 2 mg/kg/day up to 40 mg/kg, then remaining at 40 mg/kg for the last 5 days (Leathley and Goudie, 1992). On day 22, the injections of chlordiazepoxide were either continued (non-withdrawal group) or replaced with either saline or levetiracetam, 17 or 54 mg/kg (withdrawal groups). On day 23, the animals received the same treatment as on day 22, but administered 60 min before testing on the plus-maze. The dosing schedule for each group is detailed in Table 1. For testing, each mouse was placed in the centre of the maze and the number of entries onto the open arms, the time spent on the open arms and the total number of arms visited were recorded over 4 min. An entry was scored when the mouse had entered the arm with all 4 ft. The experiment was replicated, with dose-groups of nine mice and the results of the two experiments pooled to give $n=17$ per group. This replication experiment also included a group of nine mice which received saline for 22 days twice daily and levetiracetam (54 mg/kg) acutely, 60 min before testing on day 23. Further, independent groups of nine mice were treated twice daily with saline during 21 days and then received three injections of levetiracetam (17 and 54 mg/kg) or saline before testing in the elevated plus-maze on day 23. The levetiracetam-dosing schedule was similar to the one used for LEV replacement in the withdrawal study.

2.5. Statistical analysis

For each mouse, the percentage of entries onto the open arms was calculated (number of entries onto the open arms divided by the total number of entries × 100). For each treatment group, the mean and S.E.M. were calculated for the three dependent variables. The data were statistically analysed by a one-way analysis of variance (ANOVA) or the

Student's *t*-test depending on the number of groups to be compared. Post hoc Dunnett's test was performed in case of an overall significant effect of ANOVA.

3. Result

Repeated administration of chlordiazepoxide during 23 days (Fig. 1) resulted in significant increases in the time spent on open arms (Student's *t*-test, one tailed = 6.82, $P < 0.001$), the percentage of visits onto the open arms ($t = 5.07$, $P < 0.001$) and the total number of arms visited ($t = 8.66$, $P < 0.001$). Withdrawal from chlordiazepoxide (Fig. 2, group CDP–SAL) resulted in significant decrease in the time spent on open arms ($t = 4.53$, $P < 0.001$), the percentage of visits onto the open arms ($t = 4.14$, $P < 0.001$) and the total number of arms visited ($t = 4.05$, $P < 0.001$) in comparison to chronic administration of a saline solution (Fig. 2, group SAL–SAL).

Replacement of chronic chlordiazepoxide by levetiracetam (Fig. 2, groups CDP–LEV) prevented a decrease in time and percentage of visits onto the open arms ($F_{2, 48} = 4.39$ and 4.56, respectively, $P < 0.05$) as well as a decrease in the total number of arms visited ($F_{2, 48} = 5.15$, $P < 0.01$) observed in the withdrawal group (CDP–SAL). Post hoc Dunnett's test indicated that levetiracetam, 54 mg/kg but not 17 mg/kg, induced a significant effect ($p < 0.05$) on the time spent on and percentage of visits onto the open arms. Both doses significantly prevented the decreased total number of arms visited observed in the CDP–SAL group (Dunnett's test, $p < 0.05$). Furthermore, no significant difference ($P > 0.05$) was detected between the group receiving levetiracetam (54 mg/kg) in place of chlordiazepoxide (CDP–LEV 54 mg/kg) and the saline group (SAL–SAL).

Levetiracetam, 54 mg/kg given acutely to mice treated chronically with saline during 22 days, produced no significant changes ($P > 0.05$) in plus-maze exploration compared to control mice receiving only saline (Table 2). Likewise, levetiracetam, 17 and 54 mg/kg given twice at day 22 and once, 60 min before testing, at day 23 to mice having received saline during 21 days, produced no significant changes ($P > 0.05$) in plus-maze exploration compared to mice treated with saline during 23 days (Table 3).

Table 3
Effect of levetiracetam given twice daily (day 22) and acutely, 60 min before plus-maze testing (day 23), to mice treated with saline twice daily during 21 days

Group ($N = 9$)	% Visits on open arms	Time spent on open arms (s)	Total number of arms visited
Control	24 (2.4)	26 (3.6)	12 (0.5)
Levetiracetam (17 mg/kg)	23 (3.8)	25 (5.7)	13 (1.2)
Levetiracetam (54 mg/kg)	21 (3.2)	28 (3.2)	15 (1.2)

Results are means with S.E.M. in parentheses. No significant difference between group was found.

4. Discussion

Chronic administration of chlordiazepoxide for 21 days progressing in increments of 2 mg/kg, from 10 up to 40 mg/kg, twice daily, produced a significant withdrawal syndrome in the elevated plus-maze test in mice mainly characterised by decreased open arm exploration 24 h after drug discontinuation. This finding extends the results obtained by Goudie and Leathley (1990, 1995) and confirms that this specific chlordiazepoxide regime produces reliable withdrawal signs in mice as well as rats. Abrupt withdrawal from repeated administration of diazepam to rats has been reported to result in similar behavioural changes on the plus-maze (File and Andrews, 1991). One plausible interpretation is that behavioural changes reflect an anxiogenic withdrawal state. This anxiogenic effect was dose-dependently prevented by levetiracetam (17 and 54 mg/kg i.p.) administration during the withdrawal period. The higher dose, 54 mg/kg, produced significant effects on all the parameters measured. This dose is in the range of doses reported to produce significant effects in various epilepsy models (Gower et al., 1992; Klitgaard et al., 1998).

The animals treated chronically with the same chlordiazepoxide regime culminating in a dose-level of 40 mg/kg, without withdrawal, showed an increase in all the parameters recorded which was comparable in magnitude to the anxiolytic effect in the elevated plus-maze test obtained after acute administration of lower doses of 5 to 10 mg/kg chlordiazepoxide treatment in mice and rats (see, e.g. Lister, 1987; Falter et al., 1992). Tolerance to chlordiazepoxide arising from repeated exposure, may account for the similarity in anxiolytic effect elicited by the chronic high dosage compared with effective lower doses given as single injections. In the present study, the doses of levetiracetam, which opposed chlordiazepoxide-induced withdrawal signs had no effect upon plus-maze exploration in normal mice chronically administered with saline for 21 or 22 days. Thus, within the limitation of the model used, levetiracetam did not appear to possess anxiolytic activity in normal mice. The observation that it is effective against withdrawal-induced anxiety in mice chronically treated with chlordiazepoxide suggests that the anxiolytic effects of levetiracetam are dependent upon the level of stress or anxiety of the animal.

The mechanism by which levetiracetam normalises chlordiazepoxide withdrawal-induced anxiety was not explored by the present study and further studies are necessary to elucidate the neurobiological mechanism underlying its action. Several Ca^{2+} blockers like nifedipine, verapamil, diltiazem and flunarizine have been shown to be efficacious against withdrawal symptoms of benzodiazepines in rodents (Saad et al., 1997). Flunarizine has shown certain selectivity for low voltage Ca^{2+} channels (T-type) whereas the other compounds modulate high voltage Ca^{2+} channels of the L-type. In this context, it should be noted that levetiracetam is able to specifically inhibit the high

voltage Ca^{2+} channels of the N-type (Lukyanetz et al., 2001). Thus, it can be speculated from the present findings with levetiracetam that modulation of this subtype of high voltage-dependent Ca^{2+} channel may influence withdrawal-induced anxiety symptoms.

Antiepileptic drugs like sodium valproate and carbamazepine have been shown to be effective in benzodiazepine and alcohol withdrawal treatment in humans (Pages and Ries, 1998) although the precise mechanism(s) of action subserving their efficacy remain to be elucidated. However, it has recently been shown in studies using whole-cell patch-clamp techniques that levetiracetam, like carbamazepine or valproate, was able to reverse the inhibitory effects of several negative allosteric modulators of GABA-gated currents like beta-carbolines or zinc. Only a minor direct effect on GABA-gated currents was observed at concentrations beyond relevant therapeutic plasma levels in epilepsy patients (Rigo et al., 2000). Using this information coupled with the hypothesis suggesting that an endogenous benzodiazepine inverse agonist ligand is released during stressful situations, including drug withdrawal (Baldwin and File, 1988; Nutt et al., 1993), this stimulate the speculation that LEV might oppose the release of such an endogenous ligand during chlordiazepoxide-withdrawal and thereby counteract anxiety signs. This could also explain the absence of effect of levetiracetam in normal mice evaluated in the elevated plus-maze test, a situation described as involving relatively low levels of stress (Lee and Rodgers, 1991).

In conclusion, the results of the present study suggest a potential efficacy of the new antiepileptic drug levetiracetam in reducing anxiogenic effects consecutive to benzodiazepine withdrawal in humans. Further preclinical studies are needed to evaluate its efficacy on signs other than anxiety as well as in other types of withdrawal.

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