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Carbamazepine normalizes the altered behavioral and neurochemical response to stress in benzodiazepine-withdrawn rats

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Abstract

Rats chronically treated with diazepam (2 mg/kg per day, i.p.) for 21 days were tested 96 h after the last injection in both the forced swim test (inescapable stress) and in an active avoidance test (escapable stress). The influence of carbamazepine (7.5 mg/kg, i.p.) administered 25 min prior to each behavioral task was investigated. Withdrawn animals showed a reduced time spent in immobility in the forced swim test and an enhanced latency to escape in the active avoidance test. Both behavioral effects were normalized by a single carbamazepine administration. An additional experiment was performed to investigate the effect of a forced swim experience on cortical chloride uptake following GABA (γ-aminobutyric acid) stimulation 96 h after diazepam withdrawal, and the influence of a single administration of carbamazepine on these effects. An increased chloride uptake was observed in vehicle-treated rats but not in diazepam-withdrawn animals following the swimming experience. Carbamazepine pretreatment enhanced chloride uptake after diazepam withdrawal but did not modify chloride flux in stressed or unstressed vehicle-treated rats. These results support the hypothesis that diazepam withdrawal affects the ability to develop adaptive responses to stress and that carbamazepine can normalize such an alteration.

Keywords: Benzodiazepine withdrawal; Stress; GABA (γ-aminobutyric acid)-gated Cl^- flux; Forced swim; Carbamazepine; Two-way avoidance

1. Introduction

Abrupt cessation of benzodiazepine intake leads to a number of symptoms defined as the benzodiazepine withdrawal syndrome. Among others, this syndrome is characterized by anxiety, irritability, sleep disturbances, headache, palpitations, tremors, depression and increased sensitivity to sensory stimuli (Owen and Tyrer, 1983; Pertursson and Lader, 1981). Several behavioral alterations, which resemble some of the clinical withdrawal symptoms, have also been reported in animal models of benzodiazepine withdrawal. These disturbances range from spontaneous seizures, increased anxiety, weight loss, tremor and increased sensitivity to audiogenic seizures (Emmett-Oglesby et al., 1983; for review, see File, 1990). In animal models, several compounds have been reported to relieve specific behavioral disturbances resulting from benzodiazepine withdrawal. For instance, a 'peripheral' benzodiazepine antagonist (PK 11195) and a benzodiazepine antagonist such as flumazenil are both partially effective in reversing the increased anxiety and the reduced seizure threshold observable after benzodiazepine discontinuation (Miller and Koff, 1994; Byrnes et al., 1993). Additional drugs other than those acting on the GABA (γ-aminobutyric acid) receptor complex are also effective in reducing withdrawal-induced changes. For instance, compounds such as clonidine, buspirone and baclofen relieve behavioral disturbances associated with benzodiazepine discontinuation (File et al., 1991). Various strategies have been applied to humans in order to reduce the adverse effects of benzodiazepine withdrawal and to facilitate benzodiazepine discontinuation in long-term users. These strategies include a gradual dosage reduction, which usually alleviates the severity of the withdrawal symptoms (Otto et al., 1993; Schweizer et al., 1990), as well as the use of pharmacological therapy with different types of drugs such as antidepressants (Rickels et al., 1991; Ansseau and De Roeck, 1993), beta-blockers (Schweizer et al., 1991), sodium valproate (Keck et al., 1992), buspirone (Lader and

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Olajide, 1987), progesterone (Schweizer et al., 1995) and carbamazepine (Klein et al., 1986; Ries et al., 1989; Schweizer et al., 1991). Among them, antidepressants are frequently used because depressive symptoms are common after discontinuation. Beta-blockers have been selectively used to reduce palpitations and tremors; however, this group of drugs is not effective in reducing the overall incidence of withdrawal symptoms (Tyrer et al., 1981). Buspirone and progesterone have shown no significant effect in reducing withdrawal symptoms (Schweizer and Rickels, 1986; Schweizer et al., 1995). Finally, carbamazepine seems to be of some therapeutical benefit principally to those patients withdrawing from high doses of benzodiazepines (Ashton, 1994).

In a recent study, we reported that rats withdrawn from chronic diazepam administration showed behavioral and neurochemical alterations when responding to a subsequent stressful experience (Martijena et al., 1996). These disturbances were even observable when the withdrawal-induced anxiogenic behavior was no longer evident, and thus they were suggested to be an additional and critical component of the benzodiazepine withdrawal syndrome. As previously proposed (Martijena et al., 1996), the decreased ability to cope with environmental challenge could be a potential factor for further benzodiazepine consumption following benzodiazepine discontinuation. Therefore, it seems relevant to investigate compounds that could potentially normalize the disturbances observed in response to stress in benzodiazepine-withdrawn animals. Based on the assumption that benzodiazepine withdrawal affects the stress response, the goal of the present study was to test the influence of carbamazepine administration on behavioral strategies adopted by benzodiazepine withdrawn animals after exposure to either inescapable or escapable stressful situations. The behavioral procedure involving inescapable stress was a forced swim situation. When animals are forced to swim in a restricted space they initially display vigorous activity (struggling) which may reflect escape-oriented behavior (Armario et al., 1991; Molina et al., 1994); over time, rats gradually adopt an immobile posture. By assessing the rat's different behavior (struggling and immobility), the influence of the previous treatment (benzodiazepine withdrawal with or without carbamazepine pretreatment) on the behavioral strategies adopted by the animal under stressful situations can be examined. The behavioral procedure involving escapable stressors was two-way active avoidance (shuttle-box). When animals face an avoidable stressful event they first try to cope with it by performing the appropriate response, leading to escape from the aversive stimulus. In the present active avoidance paradigm, animals could escape from the nociceptive stimulus by crossing to the opposite side. By assessing the latency to escape from the shock, it is possible to evaluate the influence of benzodiazepine discontinuation associated or not with carbamazepine administration on the ability of the animals to escape from the shock event. In addition to these behavioral measurements, in the present study the effect of carbamazepine on the influence of stress exposure on the activity of cortical $GABA_A$ receptor complex was assessed by means of chloride uptake following GABA stimulation in animals with or without prior benzodiazepine withdrawal.

2. Materials and methods

2.1. Animals

Adult (220–260 g body weight) male Wistar rats were housed in groups of 4–6 per cage. Food and water were available ad libitum. Animals were maintained on a 12-h light/dark cycle (light on 7:00–19:00 h) and at a room temperature of 21–25°C.

All procedures were in agreement with the standards for the care and use of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Chronic diazepam treatment

Diazepam was chronically administered as previously described (File and Andrews, 1991). Diazepam (Roche) was suspended in water with added Tween 80 (1 drop every 2 ml). The injection volume was 1 ml/kg. Animals were daily weighed and handled, and received a daily injection of diazepam (2 mg/kg, i.p.) or vehicle for 21 days.

2.3. Carbamazepine

Carbamazepine was suspended in a vehicle solution composed of distilled water containing 2% Tween 80. The drug was administered intraperitoneally (i.p.) in an injection volume of 1 ml/kg, 25 min before each behavioral test or prior to chloride flux assays. Appropriate vehicle solutions were used for control injections (Zangrossi et al., 1992).

2.4. Experiment 1

2.4.1. Forced swim test

The forced swim test was carried out in a clear Plexiglas cylinder (18 cm diameter) containing 20 cm clean water maintained at 23°C. The number of dives, and the amount of time spent struggling and immobile were recorded over a total of 10 min. Struggling was defined as intense movement or scratching of the walls of the cylinder. A rat was judged immobile when it remained motionless, making only the necessary movements to keep its head above the water (Armario et al., 1991).

2.4.1.1. Procedure. This experiment was designed to evaluate the effect of carbamazepine on the behavior of benzodiazepine-withdrawn rats in the forced swim test. Animals

were randomly assigned to two treatment procedures: (1) chronic vehicle; (2) chronic diazepam with 96 h of withdrawal. The withdrawal time was selected based on a previous report in which anxiogenic behavior following diazepam withdrawal was no longer evident in the elevated plus-maze. Rats within each treatment procedure were distributed into two groups: vehicle or carbamazepine 7.5 mg/kg. All subjects were submitted to the forced swim test 25 min after the single administration of vehicle or carbamazepine.

2.5. Experiment 2

2.5.1. Shuttle-box testing

The escape-avoidance trial was carried out in a two-way shuttle-box (60 × 20 × 60 cm) made of Plexiglas, the floor of which consisted of stainless steel rods separated by 1.0 cm. The floor was divided into two equal-sized chambers by means of a wooden partition (1.5 cm over the grid floor). The subjects were placed in the shuttle-box and allowed 3 min to get used to the experimental environment. After that, they were submitted to 30 avoidance trials with 30-s intertrial intervals. During the first 5 s of each trial, a noise signal was activated. When the response did not occur within this period, a 1.0 mA shock was applied via the grid floor. If the response did not occur during the 15-s shock, both shock and noise signal were terminated. The escape response required was the crossing to the alternative compartment of the box. The latency (s) to escape from the footshock was recorded.

2.5.1.1. Procedure. This experiment was designed to evaluate the effect of carbamazepine on the escape performance of benzodiazepine-withdrawn rats. Animals were randomly assigned to two treatment procedures: (1) chronic vehicle; (2) chronic diazepam with 96 h of withdrawal. Rats within each treatment procedure were subdivided into two groups: vehicle or carbamazepine 7.5 mg/kg. All subjects were submitted to the shuttle-box test 25 min after the single administration of vehicle or carbamazepine.

2.6. Experiment 3

2.6.1. Microsac preparation

The cortices of two rats were pooled to provide enough tissue. Microsacs were prepared following the technique of Harris and Allan (1985). Animals were killed by decapitation and their brains were quickly removed and placed on ice. Cerebral cortices were dissected and homogenized, using a glass-Teflon homogenizer (6–8 strokes), in 8 ml of cold buffer containing (in mM): HEPES 20; NaCl 118; KCl 5; $CaCl_2$ 2.5; $MgSO_4$ 1.18 and glucose 10, adjusted to pH 7.4 with Tris base. The homogenate was centrifuged at $900 \times g$ for 15 min at 4°C. The supernatant was decanted and the pellet was resuspended in 8 ml of assay buffer and centrifuged once at $900 \times g$ for 15 min. The final pellet was resuspended in buffer, placed on ice and used immediately in the flux assays. The preparation yielded approximately 8.0–9.0 mg protein/ml of suspension. Protein content was determined by the method of Lowry et al. (1951).

2.6.2. Chloride flux assays

Aliquots (200 μl) of membranes were incubated at 30°C in a temperature-controlled water bath. After a 15-min preincubation, uptake was initiated by the addition and immediate vortexing of 200 μl buffer (also at 30°C) containing $^{36}Cl^-$ (0.2 μCi, NEN, Boston, MA, USA). Various concentrations of GABA (3–1000 μM) were added to the $^{36}Cl^-$ solution. Four seconds after the addition of $^{36}Cl^-$, influx was terminated by the addition of 3.5 ml of ice-cold buffer containing 100 μM picrotoxin, and rapid filtration under vacuum onto a 2.5-cm Whatman GF/C glass microfiber filter presoaked in 0.05% polyethyleneimine. The filter was rinsed three more times with 3.5 ml of the ice-cold picrotoxin buffer. The amount of radioactivity on the filters was assessed by liquid scintillation spectrometry. The amount of $^{36}Cl^-$ bound to the filters in the absence of membranes was subtracted from all values. Data for GABA-mediated $^{36}Cl^-$ uptake are expressed as net uptake (uptake in the presence of GABA minus basal uptake) in nmol/mg protein. Maximal stimulation by GABA of $^{36}Cl^-$ uptake and the EC_{50} for GABA stimulation were determined for each individual experiment from computer-derived curves.

2.6.2.1. Procedure. This experiment was carried out to evaluate the effect of carbamazepine on GABA-stimulated uptake of $^{36}Cl^-$ in brain cortex of rats previously treated with chronic vehicle or with chronic diazepam and subsequently confronted or not with a forced swim experience. Animals were randomly assigned to two treatment procedures: (1) chronic vehicle; (2) chronic diazepam with 96 h of withdrawal. Rats within each treatment procedure were subdivided into two groups: vehicle (vehicle/vehicle; diazepam/vehicle) or carbamazepine (vehicle/carbamazepine; diazepam/carbamazepine) 7.5 mg/kg. For all groups, rats were habituated to the manipulation that preceded their death, namely, removing them from their home cage, placing them in a guillotine for 5 s and returning them to their home cage. This procedure was repeated twice a day during chronic treatment and withdrawal prior to death. Twenty-five minutes after vehicle or carbamazepine injection, animals were left in their home cage (unstressed: vehicle/vehicle/without forced swim; vehicle/carbamazepine/without forced swim; diazepam/vehicle/without forced swim and diazepam/carbamazepine/without forced swim) or placed in the forced swim situation (stressed: vehicle/vehicle/forced swim; vehicle/carbamazepine/forced swim; diazepam/vehicle/forced swim and diazepam/carba-

mazepine/forced swim) where struggling and immobility were assessed. Animals were immediately killed for chloride flux assays.

2.7. Statistics

The behavioral effect of carbamazepine in the forced swim test was evaluated by two-way analysis of variance (ANOVA) (chronic treatment × carbamazepine). Shuttle-box testing was evaluated by three-way ANOVA (chronic pretreatment × carbamazepine × latency to escape). Chloride uptake was analyzed by three-way ANOVA (chronic pretreatment × carbamazepine × forced swim). Post-hoc comparisons were performed using the Newman-Keuls test. A P value of 0.05 or less was considered as a significant difference between groups.

3. Results

3.1. Experiment 1. Effect of carbamazepine on the behavioral response to forced swimming after 96 h of diazepam withdrawal

Fig. 1 shows the effect of carbamazepine on control rats and on those subjected to 96 h of diazepam withdrawal. Diazepam-withdrawn rats showed a reduction in the time spent immobile as compared with controls. This reduction was reversed by prior administration of carbamazepine. A two-way ANOVA based on the amount of time the animals remained motionless revealed a significant effect of the treatment ($F(1,46) = 20.43$, $P < 0.0000$), a carbamazepine effect ($F(1,46) = 30.67$, $P < 0.0000$), as well as a significant interaction between treatment and the carbamazepine effect ($F(1,46) = 16.47$, $P < 0.0002$). Newman-Keuls post-hoc test revealed that the behavior of the diazepam-withdrawn group without carbamazepine was significantly different from that of the other groups ($P < 0.01$). No differences were noticed in the number of dives or in the time spent struggling (data not shown).

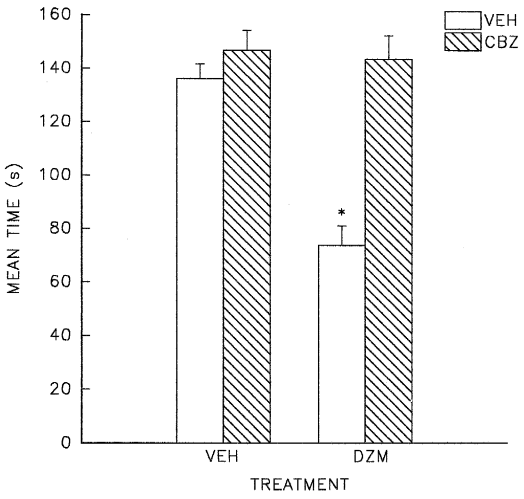

Fig. 1. Effect of carbamazepine (CBZ) on the behavior of diazepam (DZM)-withdrawn rats in the forced swim test (FS). Rats were daily pretreated during 21 days with vehicle (VEH) or DZM (2 mg/kg, i.p.). Rats were tested 96 h after the last DZM or VEH injection. Results are expressed as the mean ± S.E.M. time spent immobile (s) during 10 min of FS. Subjects were submitted to the FS 25 min after the single administration of VEH or CBZ (7.5 mg/kg, i.p.) ($n = 10$–15). * $P < 0.01$ (Newman-Keuls test) compared to the rest of the groups.

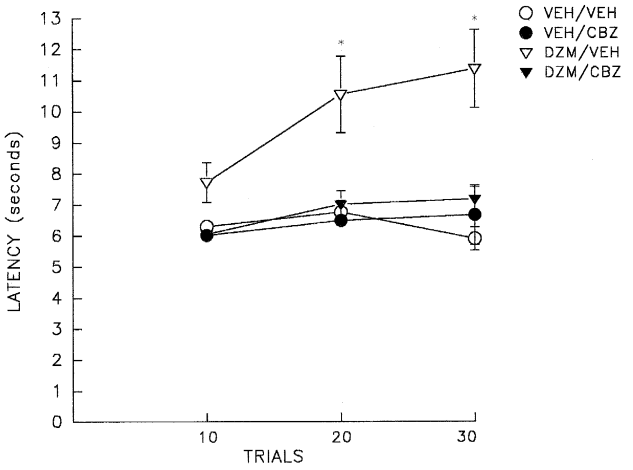

Fig. 2. Effect of carbamazepine (CBZ) on escape behavior of diazepam (DZM)-withdrawn rats in the active avoidance test. Rats were pretreated as described in the legend to Fig. 1 and behaviorally tested 96 h after the last DZM or VEH injection. Results are expressed as the mean ± S.E.M. latency to escape (s). Subjects were tested 25 min after the single administration of VEH or CBZ (7.5 mg/kg, i.p.) ($n = 8$–15). * $P < 0.01$ (Newman-Keuls test) compared to the rest of the groups.

3.2. Effect of carbamazepine on the latency to escape in a two-way active avoidance test after 96 h of diazepam withdrawal

Fig. 2 depicts that diazepam-withdrawn rats showed an increased latency to escape following 20 and 30 trials in a two-way active avoidance test as compared with control rats, and this effect was reversed by prior administration of carbamazepine. A three-way ANOVA revealed a significant interaction between pretreatment (vehicle or diazepam-withdrawn rats), treatment (vehicle or carbamazepine) and repeated measures ($F(2,74) = 3.1551$, $P < 0.0484$). Post-hoc Newman-Keuls test ($P < 0.01$) revealed that the mean escape latencies following 20 and 30 trials were longer in diazepam-withdrawn rats treated with vehicle than in rats in the other groups. No differences were observed in the percentage of avoidance in the 30-trial session. The values (mean of the percentages of conditioned avoidance response ± S.E.M.) obtained for rats vehicle/vehicle, vehicle/carbamazepine, diazepam/vehicle and diazepam/carbamazepine were 8.66 ± 3.75, 4.81 ± 2.85, 6.04 ± 2.49 and 5.45 ± 2.12, respectively.

3.3. Effect of carbamazepine on the chloride flux assay in response to a forced swim experience following 96 h of diazepam withdrawal

Fig. 3 shows an increase in the maximal chloride uptake induced by forced swim exposure in control rats. This increase was not evident in diazepam-withdrawn rats. Carbamazepine administration restored maximal chloride uptake induced by stress in diazepam-withdrawn rats. However, a slight increase in maximal chloride uptake was observed in diazepam-withdrawn rats not exposed to a swim session. No difference in basal chloride uptake measured in the absence of added GABA was observed in any experimental group (values are included in the legend to Fig. 3). A three-way ANOVA on the maximal chloride uptake values revealed no significant effect of pretreatment (vehicle or diazepam ($F(1,29) = 1.8,\ P < 0.1897$)), but a significant effect of treatment (vehicle or carbamazepine ($F(1,29) = 16.79,\ P < 0.0003$)), a significant effect of forced swim exposure ($F(1,29) = 73.193,\ P < 0.0000$), a significant interaction pretreatment × treatment ($F(1,29) = 21.08,\ P < 0.0001$), a significant interaction pretreatment × forced swim exposure ($F(1,29) = 27.34,\ P < 0.0000$), a significant interaction treatment × forced swim exposure ($F(1,29) = 9.34,\ P < 0.0048$) and no significant interaction pretreatment × treatment × forced swim exposure ($F(1,29) = 0.727,\ P < 0.400$). Subsequent Newman-Keuls post-hoc comparisons ($P < 0.01$) indicated a significant increase in maximal chloride uptake in the diazepam/carbamazepine group as compared to the diazepam/vehicle group in the same unstressed condition, and a significant difference between the diazepam/vehicle group and the diazepam/carbamazepine group for the stressed condition. Maximal chloride uptake evaluated in the stressed condition in both vehicle/vehicle- and vehicle/carbamazepine-treated rats was higher than that measured in the unstressed condition. The analysis also showed that the maximal chloride uptake of the diazepam/carbamazepine stressed group was higher than that of the diazepam/carbamazepine unstressed group.

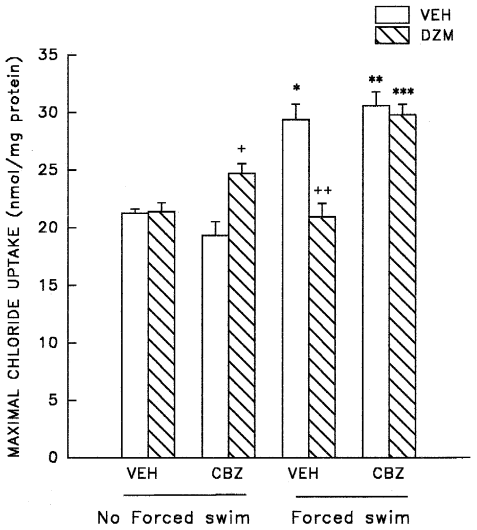

Fig. 3. Effect of carbamazepine on the chloride flux assay in response or not to a forced swim experience following 96 h of diazepam (DZM) withdrawal. Rats were pretreated as described in the legend to Fig. 1 and tested 96 h after the last diazepam injection. Animals that were not exposed to the forced swim test (NoFS) remained in the home room. Chloride uptake in cortical microsacs from subjects belonging to each experimental group was simultaneously assayed. Subjects were submitted to the forced swim test (FS) 25 min after the single administration of vehicle (VEH) or carbamazepine (CBZ) (7.5 mg/kg, i.p.). The basal chloride uptake values (in the absence of GABA) (mean ± S.E.M.) for rats vehicle/vehicle/No forced swim, vehicle/carbamazepine/No forced swim, diazepam/vehicle/No forced swim, diazepam/carbamazepine/No forced swim, vehicle/vehicle/forced swim, vehicle/carbamazepine/forced swim, diazepam/vehicle/forced swim and diazepam/carbamazepine/forced swim were 11.67 ± 1.75, 12.62 ± 1.1, 10.71 ± 1.36, 14.25 ± 0.9, 10.23 ± 0.95, 14.14 ± 1.46, 12.74 ± 1.39, 12.06 ± 1.63 nmol/mg protein respectively. Bars represent the means ± S.E.M. of maximal chloride uptake in cortical tissue (nmol Cl^-/mg of protein/4 s) of separate experiments ($n = 3–6$). For details of experimental groups see Section 2. * $P < 0.01$ VEH/VEH/FS vs. VEH/VEH/NoFS, ** $P < 0.01$ VEH/CBZ/FS vs. VEH/CBZ/NoFS, *** $P < 0.01$ DZM/CBZ/FS vs. DZM/CBZ/NoFS; + $P < 0.01$ DZM/CBZ/NoFS vs. DZM/VEH/NoFS; ++ $P < 0.01$ DZM/VEH/FS vs. VEH/VEH/FS, Newman-Keuls test.

Table 1
Effect of carbamazepine on the EC_{50} for GABA-stimulated chloride uptake in response or not to a forced swim experience following 96 h of diazepam (DZM) withdrawal

Experimental group	EC_{50} (μM) (mean ± S.E.M.)
VEH/VEH/NoFS	10.60 ± 2.56
VEH/VEH/FS	13.08 ± 2.88
VEH/CBZ/NoFS	9.87 ± 2.03
VEH/CBZ/FS	8.78 ± 0.71
DZM/VEH/NoFS	11.87 ± 2.72
DZM/VEH/FS	12.37 ± 1.50
DZM/CBZ/NoFS	21.49 ± 2.16 [a]
DZM/CBZ/FS	12.45 ± 0.50

Rats were pretreated as described in the legend to Fig. 1 and tested 96 h after the last DZM injection. Subjects were submitted to the forced swim test (FS) 25 min after the single administration of vehicle (VEH) or carbamazepine (CBZ) (7.5 mg/kg, i.p.). Animals that were not exposed to the forced swim test (NoFS) remained in the home room.
[a] $P < 0.01$ (Newman-Keuls test) compared to the rest of the groups.

As shown in Table 1, there was a significant effect of experimental pretreatment ($F(1,29) = 7.60,\ P < 0.01$) on the EC_{50} for GABA stimulation of chloride uptake, a significant interaction pretreatment × treatment ($F(1,29) = 6.57,\ P < 0.0159$) and a significant interaction treatment × forced swim exposure ($F(1,29) = 5.19,\ P < 0.030$).

4. Discussion

In support of earlier evidence (Martijena et al., 1996), when animals subjected to benzodiazepine withdrawal were

later exposed to an uncontrollable stressful situation, such as the forced swim test, the time they remained immobile was shorter. As has been tentatively suggested, immobility in this behavioral test might be the result of an adaptive response to this particular adverse experience (Armario et al., 1991; Cancela et al., 1991). Consequently, it seems reasonable to assume that withdrawn animals are less capable of developing an adaptive response to stress. Interestingly, when carbamazepine was administered prior to the forced swim experience it normalized the reduced time spent in immobility shown by withdrawn animals; however, it failed to modify the swimming behavior of control rats. Moreover, the present findings show that when animals were exposed to an aversive situation involving a nociceptive stimulus from which they could escape, rats submitted to early withdrawal had an increased latency to escape as compared with vehicle-treated animals. It is well known that benzodiazepines affect learning and memory processes and have clear amnesic effects on certain behavioral tasks (Izquierdo et al., 1990). Therefore, it could be argued that the alterations in avoidance behavior observed in withdrawn animals may reflect changes in learning and memory processing. However, considerable evidence strongly indicates that acquisition of shuttle-box avoidance is critically influenced by fearfulness (Weiss et al., 1968). In fact, exposure to an active-avoidance task seems to trigger a conflict situation, in which an increase in emotionality or fearfulness in response to shock promotes freezing (Gray, 1987; Wilcock and Fulker, 1973). This behavior is opposite to active learning to avoid shock. In support of this view, escape acquisition in an active avoidance task has been proposed as a valid model of anxiety (Gray, 1982; Fernández-Teruel et al., 1991). Furthermore, the facilitatory action on active avoidance of antianxiety agents, including benzodiazepines, is partly due to their anticonflict properties (Boix et al., 1989; Escorihuela et al., 1993). Therefore, a possible explanation for the present findings could be that withdrawn rats are more emotional in response to shock than controls. In turn, this effect could impair shuttle-box acquisition. In any case, a disturbance in escape behavior interferes with the ability to perform the appropriate adaptive behavior under this particular stressful situation. The administration of carbamazepine after withdrawal improved escape behavior since withdrawn animals previously administered carbamazepine had a similar escape behavior to that exhibited by control rats. In summary, our behavioral findings are consistent with the hypothesis that benzodiazepine withdrawal affects the ability to cope with environmental challenge, regardless of the appropriate adaptive responses – passive or active behavior – to each of these particular stressful events.

In support of previous observations (Schwartz et al., 1987; Kellogg et al., 1993; Martijena et al., 1996), exposure to a forced swim experience induced a significant increase in cortical chloride uptake following GABA stimulation in control animals; however, this effect was absent in stressed animals previously submitted to benzodiazepine discontinuation. Changes in the GABA supramolecular complex in response to stress have been consistently described by several laboratories (Drugan and Holmes, 1991). These changes seem to be mainly restricted to the chloride ionophore component of this receptor complex (Havoundjian et al., 1986a,b; Trullas et al., 1987). Moreover, the chloride ionophore is subject to tonic regulation which could be associated with the degree of stress experienced by the animals (Mason et al., 1976; Riley et al., 1981). Based on this view, it seems likely that the lack of increase in the stimulated influx of Cl^- in response to the swim experience in withdrawn rats may be related to an altered sensitivity to stressful stimuli rather than to a direct alteration of the chloride channel. Indeed, the fact that chloride uptake following GABA stimulation was similar in control and withdrawn animals in the unstressed condition supports this notion. The enhancement of GABA-stimulated chloride uptake in response to stress has been postulated as a coping mechanism associated with adaptive behavior following stressful manipulation. Based on our findings, it seems plausible to propose that benzodiazepine withdrawal may disrupt the ability to induce adaptive changes in the activity of the chloride channel. Carbamazepine administration enhanced the facilitating effect of GABA on chloride uptake in stressed withdrawn rats but did not modify stimulated chloride uptake in control stressed or unstressed rats, thus suggesting that carbamazepine may restore the capacity to induce adaptive changes in the chloride channel linked to the $GABA_A$ site in response to stress. In addition, a slight increase was observed in unstressed benzodiazepine-treated rats in response to the single administration of carbamazepine.

Altogether, the present behavioral and neurochemical data provide evidence to support the hypothesis (Martijena et al., 1996) that the altered response to both inescapable and escapable stressful situations following diazepam withdrawal could result from an inability to cope with stress and could be a potential and important component of the benzodiazepine withdrawal syndrome. Moreover, the fact that carbamazepine normalizes the behavioral disturbances in both behavioral paradigms and the altered chloride uptake following stress may indicate that this drug could have potential therapeutical properties to restore the ability to cope with stress following benzodiazepine withdrawal. In addition, and of clinical relevance, the carbamazepine dose used in the present study was lower than that usually used as anticonvulsant or anxiolytic (Zangrossi et al., 1992; Nakao et al., 1985; Almeida and Leite, 1990). Moreover, the administration of carbamazepine had no effect on animals not subjected to benzodiazepine withdrawal.

Carbamazepine is therapeutically used for the treatment of several neurologic and psychiatric disorders including certain seizures (Cereghino et al., 1974; Livingston et al.,

1974), bipolar depression (Post, 1987; Ballenger, 1988; Dalby, 1975) and trigeminal neuralgia (Bonduelle, 1976). Additional studies have shown that this drug can be effective for the treatment of ethanol and benzodiazepine withdrawal syndromes (Klein et al., 1986; Björkqvist et al., 1976). However, the neural mechanism involved in these effects has not been fully elucidated. Multiple neurotransmitter systems have been postulated to be involved in carbamazepine's central action. For instance, serotonin (Elphick et al., 1990), GABA (Galpern et al., 1991; Granger et al., 1995), noradrenaline (Purdy et al., 1977), excitatory amino acids (Lampe and Bigalke, 1990) and adenosine (Zangrossi et al., 1992) have been all linked to carbamazepine's central effects. In addition, carbamazepine modifies transmembrane cation inward currents (Yoshimura et al., 1995). It is well established that all neurotransmitters and neuromodulators in the central nervous system participate in the stress response. Consequently, and based on the present data, it is difficult to claim a unique neural mechanism by which carbamazepine could act to normalize stress responses following benzodiazepine discontinuation. Further studies are required to elucidate the neurobiological mechanism underlying this effect of carbamazepine.

In summary and regardless of the central mechanism involved in the central effects of carbamazepine administration, it is evident from the present findings that this drug may have potential properties to attenuate the behavioral and neurochemical disturbances in response to the stress associated with benzodiazepine withdrawal.

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