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Anxiolytic profile of the antiepileptic drug levetiracetam in the Vogel conflict test in the rat

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Abstract

The novel antiepileptic drug levetiracetam has been shown to reverse anxiogenic effects of benzodiazepine withdrawal in mice tested in an elevated plus-maze without altering the behaviour of normal mice in this model. This could suggest that the effect of levetiracetam is dependent upon the level of stress/anxiety of the animals. Levetiracetam was therefore further examined in another widely used animal model of anxiety, the Vogel conflict test. In the first experiment, water-deprived rats were submitted to a free drinking period (habituation) in a chamber equipped with a bottle of water. Twenty-four hours later, animals were returned to the same chamber but the licks to the water bottle were then punished with a foot shock (0.5 mA, 90 ms). In the second experiment, the procedure was modified by administering a foot shock at the end of the habituation period in order to induce a state of stress/anxiety (conditioned fear/ anticipatory anxiety) for subsequent testing. Levetiracetam (17 and 54 mg/kg) and chlordiazepoxide (5 mg/kg) were administered via the intraperitoneal route. The results indicated that in the first experiment only chlordiazepoxide showed a statistically significant anxiolytic effect. In contrast, in the second experiment, where the shock was given at the end of the habituation period, levetiracetam (54 mg/kg) revealed significant anxiolytic activity similar to chlordiazepoxide. This suggests that levetiracetam may have potential anxiolytic effects and may provide therapeutic benefits to individual with anxiety spectrum disorders.

Keywords: Anxiety; Anxiolytic effect; Vogel conflict test; Levetiracetam; Antiepileptic; Chlordiazepoxide

1. Introduction

Levetiracetam (Keppra®) is a pyrrolidine derivative, which is registered as add-on treatment of refractory partial onset seizures in adults. Its mechanism of action does not appear to involve the main cellular targets associated with classical antiepileptic drugs (Margineanu and Klitgaard, 2002). There appears to be no interactions with either voltage-activated Na^+ or T-type (low-voltage) Ca^{2+} channels in cultured neurons (Zona et al., 2001). However, levetiracetam reduces high-voltage Ca^{2+} currents in vitro (Niespodziany et al., 2001). Levetiracetam also appears to be devoid of direct GABAergic (GABA) effects (Margineanu and Klitgaard, 2002). Though, it opposes inhibition induced by negative allosteric modulators of both GABA- and glycine-gated currents (Rigo et al., 2002) and is able to reduce the hippocampal hyperexcitability produced by the $GABA_A$ receptor antagonist bicuculline, both in vitro and in vivo (Birnstiel et al., 1997; Margineanu and Wulfert, 1997). This suggests that levetiracetam might have beneficial effects in several conditions where GABA neurotransmission is altered, such as stress or anxiety disorders (Jetty et al., 2001; Lamberty et al., 2002).

We have recently shown (Lamberty et al., 2002) that levetiracetam was able to reverse anxiogenic effects of benzodiazepine withdrawal in mice tested in an elevated plus-maze test, a widely used animal model of anxiety. Interestingly, levetiracetam was without any significant effect on normal mice tested in the same model. These results might suggest that the effect of levetiracetam might be dependent upon the baseline level of stress or anxiety of the animals.

The aim of the present study was to evaluate this assumption further in another widely used animal model of anxiety, the Vogel conflict test (Vogel et al., 1971). In this procedure, the licking response for water of thirsty

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rats is suppressed if the response is punished by an electric shock. It is generally accepted that the ability of novel compounds to counteract this response constitutes a reliable parameter predictive of anxiolytic-like activity. Indeed, benzodiazepines are reported to increase the number of electric shocks supported and the amount of time spent licking. Most times, the procedure used is a modification of the original test but it rests on the principle of an unconditioned conflict situation (Pollard and Howard, 1990). Indeed, with or without a habituation session, there is no cue to signal that the licking will be punished. In the present experiments, we modified the procedure by administering a single electric shock at the end of the habituation session. An electric shock given to rats placed in a chamber during habituation (unconditioned stimulus) is supposed to induce an unconditioned response (stress, fear) and replacing the animals in the same chamber may act as a signal that the response will be punished (conditioned fear, anticipatory anxiety). Chlordiazepoxide was used as a positive control for these experiments.

2. Materials and methods

The protocol presented below was approved by the local ethics committee for animal experimentation according to Belgian law.

2.1. Animals

Two hundred and eleven male Sprague–Dawley rats (IFFA CREDO, Belgium) weighing 160–180 g were used. They were housed in groups in stainless steel standard cages. The day before testing, they were placed by groups of 5 in stainless steel cages (40 × 20 × 21 cm high). The cages were located in an air-conditioned animal holding room illuminated from 0600 to 1800 h. Food and water was given ad libitum.

2.2. Drugs and solutions

Chlordiazepoxide (Sigma) 5 mg/kg and levetiracetam (UCB S.A. Pharma Sector) 17 and 54 mg/kg were dissolved in 0.9% saline solution and administered intraperitoneally (i.p.) 60 min before the test in a volume of 5 ml/kg.

2.3. Apparatus

The test was run in sound-attenuated test chambers (Campden Instruments). The test chambers (25 × 23 × 20 cm high) were equipped with floor bars completing an electric circuit with the metal drinking spout of a water bottle fixed outside the chamber and protruding into the chamber through a hole. This system was coupled to a programmable stimulator.

2.4. Procedure

The procedure was a modification of that described by Vogel et al. (1971). In the afternoon proceeding the test day, the rats were deprived of water from 0400 PM onwards by removing the water bottles. The test was performed on two successive days. On day 1, rats were allowed to explore the chamber for 6 min and drink water ad libitum without being shocked (experiment 1: unconditioned Vogel conflict) or animals were shocked (0.5 mA for 90 ms) once, 15 s before the acquisition session was terminated (experiment 2: 'conditioned' Vogel conflict). No additional access to water was allowed to the animals afterwards. On day 2, the rats were injected i.p. with the drugs or saline 60 min before the test. For testing, the rats were returned to the test chamber and allowed to drink freely for 3 s. At the end of this interval, they received their first shock (0.5 mA for 90 ms). From this moment on, the animals received a shock each time they had summed up a total time of contact with the water spout of 3 s. Automated records were taken of the latency with which the animals started drinking, the total time they spent on drinking and the total number of shocks they received. The test session lasted for 4 min. To control the effect of the shock given during the habituation session on subsequent testing, we included a further control group in experiment 2. This group did receive a shock on day 1 (habituation) but none on day 2. Performance was compared with the control group that did not receive any shock either on day 1 or day 2. To control for effects on nociception, separate groups of water-deprived animals were evaluated for their sensitivity to electric shocks in the same type of chamber. Both levetiracetam and chlordiazepoxide were given i.p., 60 min before the test. During testing, animals were allowed 2 min to explore after which 90 ms electric shocks presented in increment of 0.1 mA were delivered at 15-s intervals. The shock intensities producing a vocal and flinch response were recorded.

2.5. Statistical analysis

Results are presented in terms of mean with S.E.M. The Bartlett test indicated that the variances were not significantly different between the groups. Therefore, the results were statistically analysed with the Student's *t*-test or a one-way analysis of variance (ANOVA) depending on the number of groups to compare. A post hoc Dunnett test was applied in case of an overall significant effect of ANOVA.

3. Results

3.1. Experiment 1: unconditioned Vogel conflict test

The results are presented in Fig. 1. ANOVA indicated an overall effect of drugs for both the time spent licking and the

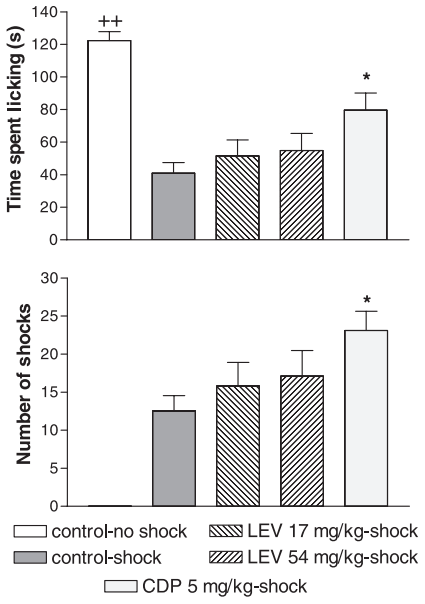

Fig. 1. Effect of levetiracetam 17 and 54 mg/kg and chlordiazepoxide 5 mg/kg on time spent on licking (upper panel) and number of shocks received (lower panel) by water-deprived rats receiving electric shocks (0.5 mA; 90 ms) or controls evaluated in the Vogel test ($n=17$/group). All rats were submitted to a habituation session of free drinking, without shock, in the same chamber 24 h earlier. Results are expressed in terms of mean ± S.E.M. *$P<0.05$ (Dunnett test); $^{++}P<0.01$ (Student's *t*-test) with regard to control animals receiving shocks.

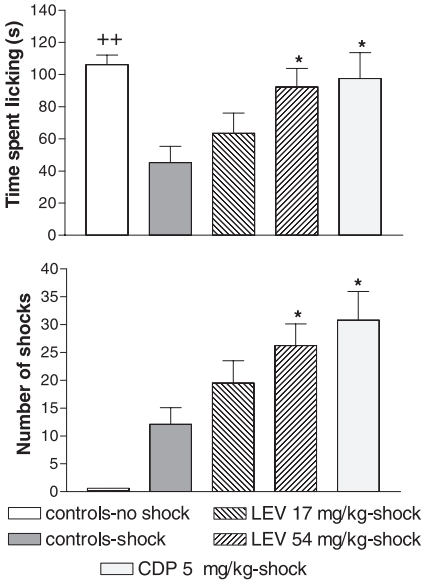

Fig. 2. Effect of levetiracetam 17 and 54 mg/kg and chlordiazepoxide 5 mg/kg on time spent on licking (upper panel) and number of shocks received (lower panel) by water-deprived rats receiving electric shocks (0.5 mA; 90 ms) or controls evaluated in the Vogel test ($n=17$/group). All rats were submitted to a habituation session of free drinking in the same chamber 24 h earlier. They received a single electric shock 15 s before the end of the habituation session. Results are expressed in terms of mean ± S.E.M. *$P<0.05$ (Dunnett test); $^{++}P<0.01$ (Student's *t*-test) with regard to control animals receiving shocks.

Table 1
Consequence of an electric shock given at the end of the habituation session on subsequent testing in water-deprived control animals

Condition		Latency to start drinking (s) on day 2	Time on licks (s) on day 2
Day 1	Day 2		
No shock	no shock	4.7 (1.4)	106.1 (5.9)
A single shock	no shock	10.4 (2.7)[a]	101.0 (7.3)

Results are expressed in terms of mean with S.E.M. in parentheses.
[a] $P<0.05$, Student's *t*-test ($n=17$/group).

number of shocks received ($P=0.03$). However, post hoc Dunnett test indicated that only chlordiazepoxide significantly increased the time spent on licking as well as the number of shocks received ($P<0.05$) with levetiracetam showing a trend towards increasing both parameters. As expected, control animals that did not receive electric shocks spent significantly more time on licking than controls receiving shocks during testing (Student's *t*-test, $P<0.01$).

3.2. Experiment 2: 'conditioned' Vogel conflict test

The results are presented in Fig. 2. ANOVA indicated an overall effect of the drugs tested for both parameters ($P=0.01$). Post hoc Dunnett test indicated that both chlordiazepoxide and levetiracetam (54 mg/kg) significantly increased the time spent on licking and the number of shocks received ($P<0.05$). Control animals that did not receive electric shocks spent significantly more time on licking than controls receiving shocks during testing (Student's *t*-test, $P<0.01$). The effect of a single electric shock given at the end of the habituation period on subsequent testing was observed to significantly increase the latency with which the animals started to drink during testing on day 2 ($P<0.05$, Table 1).

3.3. Electroshock sensitivity

The results of sensitivity to the electric shock are presented in Table 2 and indicate that levetiracetam 17 or 54 mg/kg did not significantly alter the reaction to electric shocks as measured by vocalisations or flinch responses.

Table 2
Reactivity threshold to electric shock

Mean shock intensity with S.E.M. (mA)	Vocal response	Flinch response
Controls	0.40 (0.03)	0.21 (0.01)
Levetiracetam 17 mg/kg	0.41 (0.03)	0.23 (0.02)
Levetiracetam 54 mg/kg	0.50 (0.04)	0.28 (0.03)

Water-deprived rats ($n=8$/group) were placed on an electrified grid floor after which 90-ms electric shocks presented in increment of 0.1 mA were delivered at 15-s intervals. The shock intensities producing a vocal response and flinch response were noted.

4. Discussion

The present study suggest that levetiracetam possesses anxiolytic activity in a modified version of the Vogel conflict test. This effect was statistically significant at a dose of 54 mg/kg. The same dose was also noted in previous experiments to completely reverse the anxiogenic effect, measured in an elevated plus-maze test, of chlordiazepoxide withdrawal in mice (Lamberty et al., 2002). This dose is in the range of doses that showed activity in epilepsy models in mice and rats (Klitgaard et al., 1998) as well as in a putative animal model of mania in the rat (Lamberty et al., 2001).

Levetiracetam has been shown to be devoid of any anxiolytic effect in a standard elevated plus-maze test in the mouse (Lamberty et al., 2002) and the present results also confirm that in a widely used procedure of the Vogel conflict test, i.e. unconditioned conflict situation, levetiracetam did not induce statistically significant anxiolytic effects, although a trend was noted at the highest dose tested. In contrast, chlordiazepoxide significantly increased both the time spent on licking and the number of shocks received at a dose of 5 mg/kg, showing that the experimental conditions were sensitive to the anti-conflict effect of benzodiazepines as reported in the literature (Treit, 1985).

In the second experiment of the present study, an electric shock was applied at the end of the habituation period in order to change the state of the animal for subsequent testing 24 h later. This procedure to a certain extent mimics a conditioned fear since the test chamber could act as a signal that licking will be punished. Interestingly, the consequences of this change made on day 1 did not appear to impact the time animals spent licking or the number of shocks received by controls animals during retesting on day 2. Further analysis however, indicated that the latency with which control animals started to drink on day 2 was significantly increased in the group that received an electric shock at the end of the habituation session on day 1. This indicates that animals were probably more anxious and hesitate to start drinking, something predicted from the change in procedure. As for the effect of drugs, levetiracetam, at a dose of 54 mg/kg, significantly increased the time spent licking as well as the number of shocks received, thus resulting in a profile comparable to chlordiazepoxide. The latter drug also produced a more marked effect in the second experiment compared to the first one, albeit to a lesser extent than that observed to levetiracetam.

Finally in a parallel experiment, shock thresholds were measured in animals to control for any effect on nociception with the drugs used. Results indicated that treatment with levetiracetam did not alter the drug-induced sensitivity of water-deprived rats compared to controls. Therefore, the effect of levetiracetam in this procedure could be ascribed to an anxiolytic rather than an analgesic effect. Together, these results confirm that benzodiazepines are effective in conditioned and unconditioned fear procedures (Treit, 1985; Pollard and Howard, 1990) and show that levetiracetam discriminates between the two protocols. The neural mechanisms in the conditioned fear procedure are likely to be different from those involved in the classical unconditioned conflict situation (Schulkin et al., 1994; Korte, 2001). In the conditioned procedure, a cognitive appraisal of threat is supposed to induce a different emotional state than in the classical unconditioned situation. Moreover, the procedure involving fear conditioning might be considered as a more relevant model of pathological anxiety because an enhanced anxiety state is involved in many psychopathologies (Korte, 2001; LeDoux, 1995).

Traditional unconditioned conflict tests have been used with success to screen compounds that interact with the GABA/benzodiazepine receptor complex (Treit, 1985). However, atypical anxiolytics such as the 5-hydroxytryptamine (5-HT) 1a receptors ligands are generally not detected in the Vogel conflict test (Griebel, 1995). Thus, it is no surprise that levetiracetam which does not interact directly with the GABA/benzodiazepine receptor complex (Margineanu and Klitgaard, 2002) is lacking activity in this classical situation. In this respect, it should be interesting to evaluate, for example, the effect of 5-HT 1a receptor agonists buspirone or ipsapirone in our modified version of the Vogel test. If benzodiazepines are effective in conditioned and unconditioned fear procedures, the mechanism by which they modify behaviour is not necessarily the same. Behavioural disinhibition and memory (acquisition, storage or retrieval) disruption rather than true anxiolytic effects has repeatedly been advanced to explain the effect of benzodiazepines in unconditioned and conditioned conflict situations respectively (Dantzer, 1987; Pain et al., 2002). Thus, the present results indicate that it is unlikely that levetiracetam produced behavioural disinhibition because it is not effective in the classical procedure. Moreover, it is unlikely that levetiracetam impact retrieval of contextual fear conditioning due to its pro-cognitive properties (Verloes et al., 1988) or absence of negative impact on cognition in tests sensitive to benzodiazepines (Verloes et al., 1988; Lamberty et al., 2000).

Other antiepileptic drugs have been tested in animal models of anxiety with variable results. Amongst them, sodium valproate and gabapentin were shown to have anxiolytic activity in a standard elevated plus-maze and in conflict tests in the rat (File and Aranko, 1988; Singh et al., 1996; De-Paris et al., 2000; Dalvi and Rogers, 2001). This deviation from our results may relate to the different mechanism of action of these drugs. In this respect, it is worth noting that, contrary to levetiracetam, gabapentin, for example, appears to have direct effects on GABA concentrations, GABA turnover and GABAergic nerve function (Moshé, 2000; Taylor, 2002). The fact that leve-

tiracetam opposes the action of both the $GABA_A$ receptor antagonist bicuculline and beta-carbolines (Rigo et al., 2002) without significantly altering GABA-elicited currents suggest that the action of levetiracetam is dependent on a deficient GABAergic function or at least a physiological/behavioural state that could impact GABAergic neurotransmission. At a behavioural level, it is difficult to measure overt effect of levetiracetam when normal rodents are used. However, when ''pathological'' animals are used, such as rats with genetic epilepsy, sound-sensitive mice, cognitively impaired mice (Gower et al., 1995, Klitgaard et al., 1998, 2002) or chlordiazepoxide-withdrawn mice (Lamberty et al., 2002), the effect of levetiracetam are clearly expressed. This could explain the lack of activity of levetiracetam using normal animals in an elevated plus-maze, a situation often described as involving relatively low levels of stress (Lee and Rodgers, 1991). This could also explain why levetiracetam shows clear-cut anxiolytic activity in the 'conditioning' procedure of the Vogel test rather than in the standard one. Related to this, several studies have suggested that endogenous inverse agonist ligands for the benzodiazepine receptor are released during fear conditioning (Izumi et al., 1999). This stimulates the speculation that levetiracetam might oppose the release of such an endogenous ligand in the conditioned procedure of the Vogel test.

Clearly, further studies are warranted to directly compare the physiological consequences of the two different procedures in the rat and to better understand the differential effect of levetiracetam in these procedures.

In conclusion, the present study indicates that in a modified procedure of the Vogel conflict test mimicking a contextual fear-conditioning procedure, levetiracetam produces comparable effects to chlordiazepoxide that can be ascribed to an anxiolytic activity. These results also suggest that the effect of levetiracetam is dependent on the emotional state of the animals and that this antiepileptic drug may have beneficial effects in certain human anxiety disorders.

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