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Effects of Valproate and Lorazepam on Experimental Anxiety: Tolerance, Withdrawal, and Role of Clonidine

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DE ANGELIS, L. *Effects of valproate and lorazepam on experimental anxiety: Tolerance, withdrawal, and role of clonidine.* PHARMACOL BIOCHEM BEHAV **52**(2) 329–333, 1995. – The anxiolytic-like effects tolerance and withdrawal from chronic treatment with sodium valproate [200, 300, and 400 mg/kg, intraperitoneally (IP)] were compared with those of a known anxiolytic drug, lorazepam (0.025, 0.05, and 0.10 mg/kg, IP), in the light–dark aversion test in mice. Furthermore, we investigated whether acute treatment with clonidine, 0.03 mg/kg, IP, an α2-adrenoceptor agonist, could reduce the increased anxiety on withdrawal from chronic treatment. Mice were given 14 daily IP injections of valproate, lorazepam, or vehicle and were tested in the light–dark aversion test 30 min or 24 or 48 h after the last drug or vehicle administration. Results showed that both acute and chronic valproate treatment reduced the aversion of mice for the light area, as well as increased the number of transitions, thus indicating an anxiolytic-like potential. Furthermore, in contrast to lorazepam, tolerance to the anxiolytic-like effects of valproate did not occur, and withdrawal from chronic treatment (300 mg/kg, IP) in our behavioral paradigm was not associated with any behavioral disturbances referring to an increased anxiety state. Finally, low doses of clonidine (0.03 mg/kg, IP) were shown to have anxiolytic properties and to reverse the anxiogenic effects of lorazepam on withdrawal.

Valproate Lorazepam Clonidine Withdrawal Mouse Light–dark box

PREVIOUS animal (2,3,21) and clinical (14,16,17) research provided evidence that the anticonvulsant drug sodium valproate (VPA) may display benzodiazepine (BDZ)-like effects on experimental anxiety. Because a major problem in the therapeutic use of BDZ is the development of tolerance, dependence, and withdrawal syndrome following cessation of chronic treatment, it is useful to identify anxiolytic agents that lack these properties. In this connection, the majority of research has focused on the activity of agents that enhance γ-aminobutyric acid (GABA)-ergic neurotransmission (i.e., VPA) (18,20).

The purpose of the present experiment was to determine whether it is possible to detect tolerance to the anxiolytic-like effects of VPA and overt behavioral disturbances following cessation of chronic administration with VPA. For comparison, it was considered interesting to include a BDZ derivate, lorazepam (LOR). Furthermore, because considerable amount of pharmacologic evidence (11) suggests that the noradrenergic (NA) system may be involved in fear and anxiety, studies were carried out to determine the effects of an α_2-adrenoceptor agonist, clonidine (CLO), on experimental anxiety. In particular, the investigation focused on whether CLO could reduce increased anxiety on withdrawal from chronic treatment with BDZ. The test of anxiety selected was the light–dark aversion (5) and was carried out in mice.

METHOD

Animals

Female Charles River CD1 mice (60 days at the time of the experiments, weighing 22–24 g) were used. They were acclimatized to laboratory conditions for 1 week before the start of the study, and were housed in groups of 10 in opaque polycarbonate boxes (27 × 21 × 14 cm) under standard laboratory conditions (relative humidity 50–60%, temperature 21 ± 2°C) with free access to food and tapwater. Mice were maintained on a controlled lighting cycle (dark period 0700–1900 h) for 14 days before the beginning of drug administration.

Drugs

The following drugs were used: sodium valproate (Depakin®; Labaz, Paris, France/Sigma Tau, Rome, Italy): 200,

300, and 400 mg/kg; lorazepam (Tavor®; Wyeth, Aprilia, Italy): 0.025, 0.05, and 0.10 mg/kg; clonidine (Catapresan®; Boehringer Ingelheim, Firenze, Italy): 0.03 mg/kg. All quantities of drugs in the text refer to the weight of the drug as the salt or base where appropriate. Our doses were chosen from preliminary dose-response experiments or our previous data (1-3). VPA and LOR solutions were obtained by diluting the commercial solutions in distilled water. Appropriate vehicle solutions were used for control injections. In experiments using vehicle administrations, preliminary studies indicated that the responses of vehicle-injected and nontreated mice were indistinguishable, and in the following results only the response of vehicle-treated animals is given. All drugs or vehicle were administered intraperitoneally (IP) in a volume of 0.01 ml/g body wt. The drugs were coded so that the observer scoring the test had no knowledge of the drug treatment of any mouse.

Apparatus

The light-dark aversion test was performed according to Belzung et al. (5). The apparatus consisted of two polycarbonate boxes (27 × 21 × 14 cm) with an interconnecting black plastic tunnel (7 × 10 cm). One of these boxes was painted black and topped with a black cover. The other box was lit by a 60-W desk lamp 30 cm above the box, which provided the only laboratory illumination. The apparatus was positioned on a bench 1 m above the floor.

Procedure

Testing was carried 30 min after acute dosing, 30 min after the last administration on the 14th day of treatment, and 24 and 48 h after withdrawal from chronic treatment with VPA, LOR, or vehicle. CLO or vehicle was administered 30 min before testing in mice withdrawn from chronic treatment with LOR. Each mouse was placed into the center of the lit box. During the 5-min test, the number of transitions between the lit and dark areas and the time in the lit area was determined, minute by minute, by an observer in another room via a closed-circuit TV camera. A mouse was considered to have entered the new area when all four legs were in this area. None of these animals was used on more than one occasion. All tests were run between 0900 and 1200 h in a room shielded from laboratory sounds. At the end of each session, any boluses were removed and the floor of the box was wiped with detergent and dried.

Statistical Evaluation

Results are expressed as mean ± SEM. The measures, number of transitions, and time spent in the lit area/5 min were analysed by one-way analysis of variance (ANOVA) using Pharmacological Calculation System software (25). Where appropriate, comparisons between individual groups were made with Newman-Keuls test.

RESULTS

Effects of VPA Given Acutely, Chronically, or Withdrawn From Chronic Treatment

In the VPA groups (Fig. 1a), ANOVA revealed a significant effect of tretment [$F(2, 99) = 7.55$, $p < 0.01$] with post hoc testing indicating that only groups treated acutely with VPA 400 mg/kg differed significantly from the controls ($p < 0.01$). When administered chronically, VPA still produced a significant increase in this parameter, and all the groups receiving 200, 300, and 400 mg/kg had means significantly greater than the controls ($p < 0.01$, Newman-Keuls test). When mice were tested 24 h after the last IP injection with VPA 300 mg/kg, there was a significant increase in the number of transitions compared to controls ($p < 0.01$, Newman-Keuls test). In contrast, after 48 h withdrawal from VPA 300 mg/kg treatment, there was no significant difference from the controls.

Figure 1b shows that VPA reduced the aversion of mice for the light area of the test box [$F(2, 99) = 97.63$, $p < 0.01$]. Post hoc Newman-Keuls test showed that groups treated acutely with 300 and 400 mg/kg VPA differed significantly from the controls ($p < 0.01$). Similarly, this aversion was decreased significantly by chronic administration of VPA 200, 300, and 400 mg/kg or 24 h ($p < 0.01$, Newman-Keuls test) or 48 h ($p < 0.05$, Newman-Keuls test) after withdrawing from VPA 300 mg/kg. Furthermore, there were no significant differences in either measure of the light-dark aversion test between mice given acutely and those given chronic VPA treatment, suggesting no development of tolerance.

Effects of LOR Given Acutely, Chronically, or Withdrawn From Chronic Treatment

An ANOVA performed on data presented in Fig. 2a and b revealed that the effect of LOR on the number of transitions [$F(2, 82) = 31.47$, $p < 0.01$] and the time spent in the lit area [$F(2, 82) = 44.39$, $p < 0.01$] was significant. Further statistical analysis indicated that only groups treated acutely with 0.05 and 0.10 mg/kg LOR differed significantly ($p < 0.01$) from the controls in increasing the number of transitions (Fig. 2a) or the time spent in the lit area (Fig. 2b).

Given chronically, LOR induced a similar pattern of results (Fig. 2a), but only the 0.05-mg/kg groups differed significantly from the controls in increasing the time spent in the lit area ($p < 0.01$, Newman-Keuls test) (Fig. 2b).

Furthermore, in contrast to VPA, there was tolerance to the anxiolytic effect of LOR such that mice chronically treated with LOR (0.05 and 0.10 mg/kg) (Fig. 2a) and LOR (0.10 mg/kg) (Fig. 2b) showed a significant reduction in both parameters of the light-dark aversion test compared with acutely treated mice ($p < 0.01$, Newman-Keuls test). A comparison between control mice and mice withdrawn 24 or 48 h from chronic LOR 0.05 mg/kg resulted in a significant decrease in both measures of the light-dark aversion test, thus indicating an increased anxiety state (Fig. 2a and b) ($p < 0.05$, Newman-Keuls test). The administration of CLO 0.03 mg/kg failed significantly to reverse the decrease in the number of transitions, at both 24 and 48 h withdrawal (Fig. 2a), but significantly ($p < 0.05$) increased the time spent in the lit area (Fig. 2b), thus indicating a reduction in the aversive response to the light environment and referring to an anxiolytic effect.

DISCUSSION

The present data confirm previous ones using a variety of behavioral tests [conflict test (15), antagonism of pentylenetetrazole discrimination (19), staircase test (24), hyponeophagia (22), elevated plus-maze (10), social interaction (6), light-dark aversion test (2,3)] that demonstrate anxiolytic-like effects of VPA on experimental anxiety.

Therefore, the present findings provide an important indication of the ability of VPA to antagonise anxiety-related behavior in humans. In fact, several clinical studies (14,16,17)

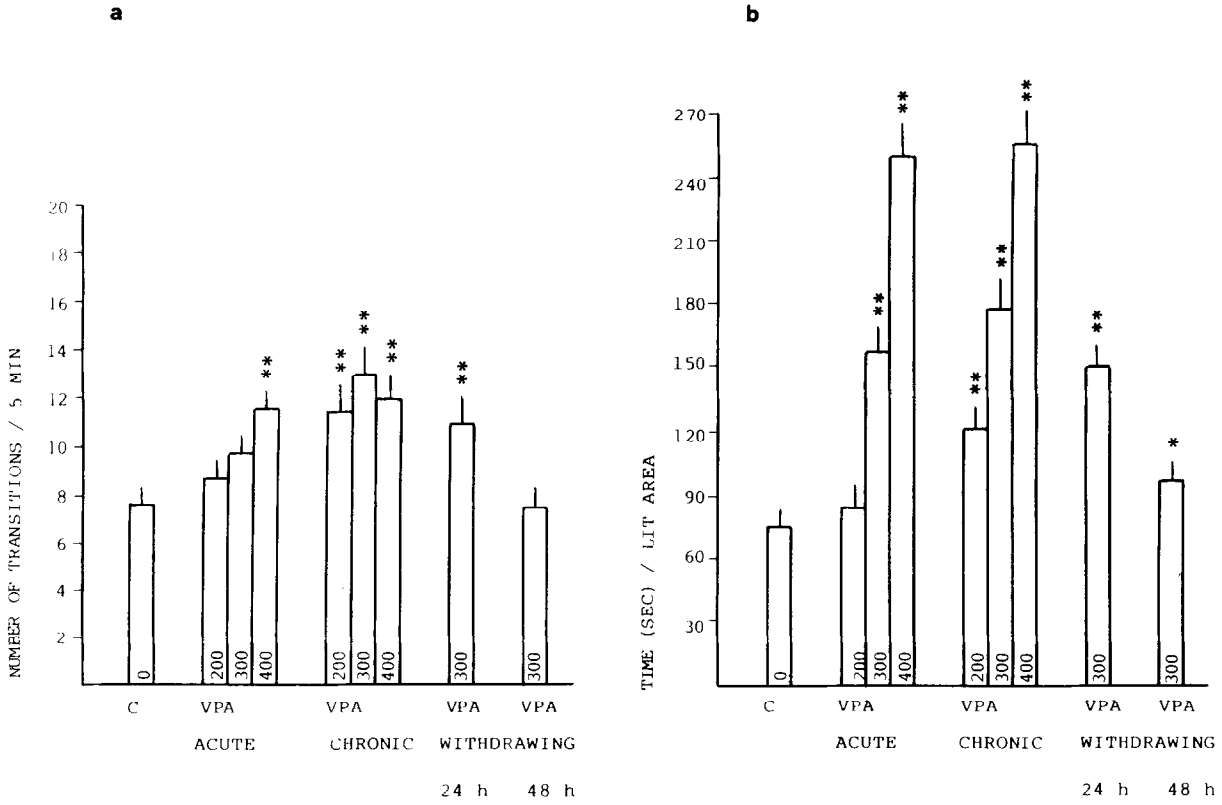

FIG. 1. The effects of sodium valproate (VPA) 200, 300, and 400 mg/kg, IP, given acutely, chronically or withdrawn from chronic treatment, in the light-dark aversion test in mice. Testing was carried out 30 min after acute dosing, 30 min after the last dose on the 14th day of treatment, and then 24 and 48 h after withdrawal from chronic treatment with VPA or vehicle. As there was no significant difference in behavioral parameters between control (C) mice receiving acute, chronic, or withdrawal vehicle (0) treatment, the means of these groups have been combined. The number of transitions (a) between the lit and dark areas and the time spent in the lit area (b) were scored over 5 min. Data are expressed as the mean ± SEM (n = 6, except C = 12) *$p < 0.05$; **$p < 0.01$, significantly different from controls. One-way ANOVA, post hoc Newman-Keuls test.

reported that VPA may be effective in the treatment of panic disorders. However, hypotheses on the site and mechanisms of action of VPA to achieve such effects remain unclear. The anxiolytic effect of VPA within the brain could be correlated with the VPA-induced increase in GABA levels in various brain regions via inhibition of GABA catabolism and activation of GABA synthesis (23). In fact, it is known that drugs that enhance GABA-ergic neurotransmission (e.g., BDZ) exert anxiolytic effects (20).

From the data on LOR, it is clear that, like VPA, LOR displayed a marked anxiolytic profile. As compared to animals treated acutely, animals treated chronically with LOR, in contrast to VPA, showed a decrease in both measures of the light-dark aversion test, suggesting tolerance to the disinhibitory properties of LOR. Taken together, these data confirm the validity of the light-dark aversion test as an animal model of anxiety in mice (7), because other studies (8,9) in these experimental conditions showed tolerance and withdrawn anxiety in mice treated chronically with BDZ. A further important difference between VPA and LOR was revealed on withdrawal from chronic treatment. Withdrawal from treatment with VPA was not associated with an increased anxiety state referred to as withdrawal anxiety.

In contrast to VPA, behavior consistent with a such withdrawal anxiety was demonstrated to occur after cessation of chronic administration with LOR, as shown by an increased aversion of mice to light.

In this context, another aspect of this study deserves some comment—that is, the effects of CLO in mice withdrawn from chronic LOR treatment. Our results show that low doses of CLO (0.03 mg/kg) may have anxiolytic properties and are able to reverse anxiogenic effects (i.e., increased aversion to the light area) of LOR withdrawal. In particular, other studies (12) have shown that the anxiolytic-like activity of CLO was evident over a very narrow dose range: Increasing the dose (0.075 mg/kg, IP) resulted in an anxiogenic-like profile. According to these findings, a previous study (1) confirmed that an anxiolytic effect is seen at doses of 0.03 and 0.06 mg/kg, IP. In fact, these doses induced a significant increase in the time spent in the lit area without influencing the number of transitions in the blacklit area. These data indicate that CLO does not increase spontaneous motor activity. The reversal of the anxiolytic effect of CLO with increasing doses supports the clinical observation that CLO may make anxiety worse in some groups of patients (13).

The ability of low doses of CLO, an α_2-adrenoceptor agonist, to reverse the anxiogenic response significantly during LOR withdrawal suggests that in these experimental condi-

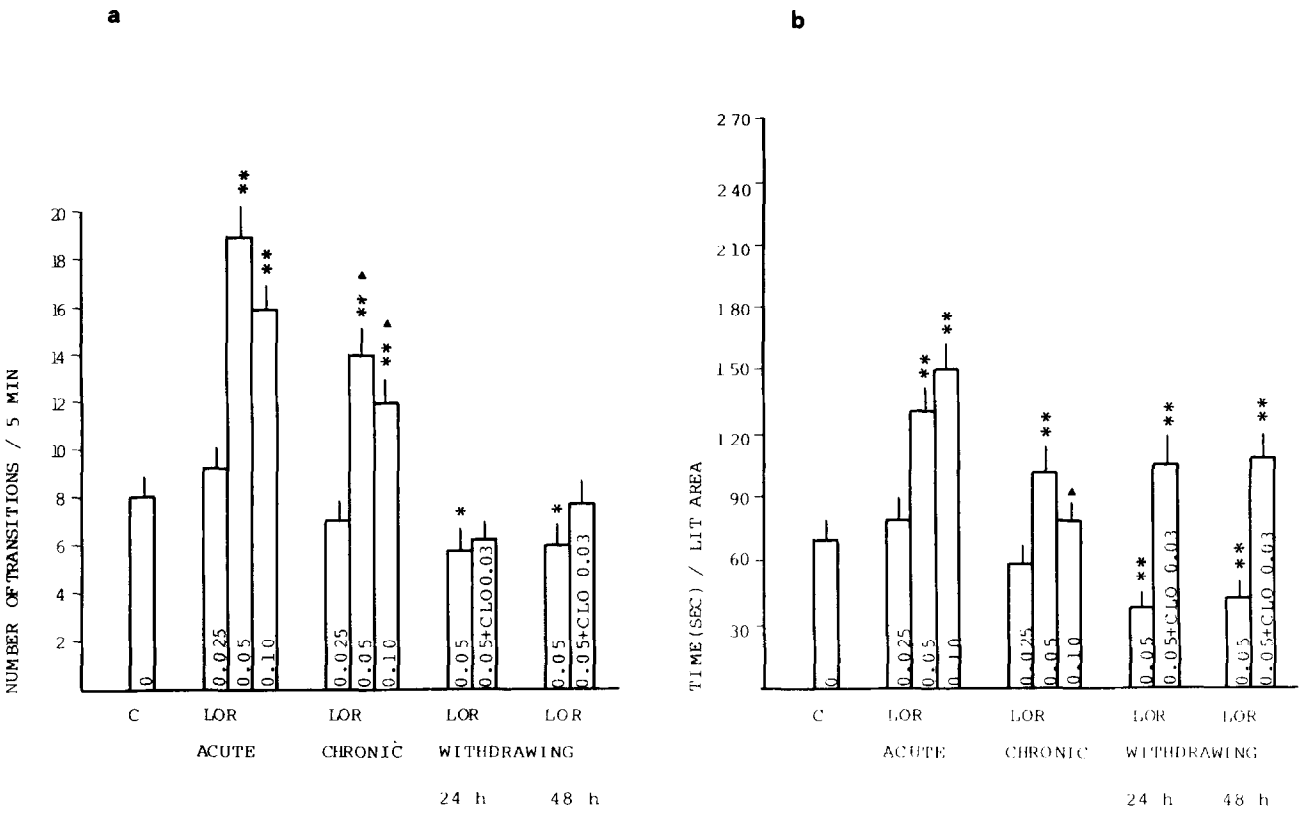

FIG. 2. The effects of lorazepam (LOR) 0.025, 0.05, and 0.10 mg/kg, IP, given acutely, chronically, or withdrawn from chronic treatment with LOR. Clonidine (CLO) 0.03 mg/kg, IP, or vehicle (0) was administered 30 min before testing in mice withdrawn (24 and 48 h) from chronic treatment with LOR (0.05 mg/kg, IP). As there was no significant difference in behavioral parameters between control (C) mice receiving acute, chronic, or withdrawal vehicle (0) treatment, the means of these groups have been combined. The number of transitions (a) between the lit and dark areas and the time spent in the lit area (b) were scored over 5 min. Data are expressed as the mean ± SEM (n = 6, except C = 12) *$p < 0.05$; **$p < 0.01$, significantly different from controls; ▲ $p < 0.01$, significantly different from acute LOR. One-way ANOVA, post hoc Newman-Keuls test.

tions, unlike those tested by Baldwin et al. (4) in the rat, there was evidence of NA involvement in the anxiogenic BDZ-withdrawal response.

In conclusion, these data provide evidence that in the behavioural paradigm used, VPA treatment may have anxiolytic-like effects without inducing tolerance and consequences of withdrawal. Further investigations will be needed to determine whether the potential anxiolytic agent VPA can cross tolerate with BDZ and make its substitution possible for BDZ treatment in humans.

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