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Short communication

Picrotoxin blocks the anxiolytic- and panicolytic-like effects of sodium valproate in the rat elevated T-maze

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Abstract

The effect of acute sodium valproate administration, an anxiolytic and putative panicolytic drug, was evaluated in rats tested in the elevated T-maze, an animal model that measures two defensive reactions: avoidance (inhibitory avoidance), related to generalized anxiety, and escape (escape from open arms), related to panic. Additionally, the involvement of γ-aminobutyric acid (GABA) neurotransmission in sodium valproate effects was studied by picrotoxin co-administration. Sodium valproate (300 mg/kg, intraperitoneally, 30 min before the test) impaired both avoidance latency (time to leave the closed arm) and one-way escape (latency to enter the closed arm) indicating anxiolytic and panicolytic effects, respectively. Pre-treatment with picrotoxin (0.5 mg/kg, intraperitoneally, 5 min before sodium valproate administration) blocked the effects of sodium valproate on inhibitory avoidance and one-way escape. No locomotor effect was seen in the open-field. These data suggest that sodium valproate exerts anxiolytic-like and panicolytic-like effects in the elevated T-maze and that these effects were mediated by picrotoxin-sensitive GABA type A receptors.

Keywords: Animal model; Anxiety; Anticonvulsant drug; Panic; (Rat)

1. Introduction

Sodium valproate is an anticonvulsant drug that exhibits an anxiolytic-like effect on animal models of anxiety in rats (Lal et al., 1980; Petersen and Lassen, 1981; Bovier et al., 1982; Myslobodsky et al., 1983; Shephard and Estall, 1984; Vellucci and Webster, 1984; Guy and Gardner, 1985; Shephard et al., 1985, 1990; Shephard, 1988; Corbett et al., 1991; Barros et al., 1992) and mice (Simiand et al., 1984; de Angelis, 1992a,b, 1995; Dalvi and Rodgers, 1996, 2001; Lang and de Angelis, 2003), although negative results were also found (File and Aranko, 1988; Mirza et al., 2005). However, although there are clinical data suggesting a putative antipanic effect (Lum et al., 1990; Primeau et al., 1990; Keck et al., 1993; Woodman and Noyes, 1994; Baetz and Bowen, 1998), there are no clinical studies evaluating the putative anxiolytic effect of sodium valproate in generalized anxiety disorder.

The behavioral effects of sodium valproate appear to be related to increased γ-aminobutyric acid (GABA) transmission, by a GABA-transaminase inhibition, decreased glutamatergic transmission, and the blockade of voltage-dependent sodium dependent currents (Loscher, 2002). Picrotoxin, a noncompetitive GABA type A receptor antagonist, blocked the anxiolytic-like effect of valproate (Liljequist and Engel, 1984; Vellucci and Webster, 1984; Shephard et al., 1985, 1990; Dalvi and Rodgers, 2001). These results suggest that the anxiolytic effect of valproate is related to GABA type A receptors. Moreover, they showed that picrotoxin is a useful tool for studying GABAergic mediation. However, only one study (Dalvi and Rodgers, 2001) tested whether the picrotoxin doses used were intrinsically active (i.e., exert an anxiogenic effect when given alone).

The elevated T-maze, which is derived from the elevated plus-maze by closure of one enclosed arm, was proposed as an animal model for measuring two types of defensive reactions related to

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anxiety: escape and avoidance (Graeff et al., 1993, 1998; Zanoveli et al., 2005). The former may be related to panic anxiety and was measured by timing the latency of escaping from one open arm (one-way escape). Avoidance behavior, which may be related to generalized anxiety disorder, was measured by the increase in latency to leave the closed arm in three successive trials (inhibitory avoidance). A number of studies report the pharmacological validation of the elevated T-maze. Inhibitory avoidance was impaired by drugs that are effective in the treatment of generalized anxiety disorder, such as diazepam, buspirone, ritanserin, chronic imipramine and paroxetine. The one-way escape time was increased by effective antipanic treatments (chronic imipramine, paroxetine, clomipramine and fluoxetine); and clinically ineffective antipanic drugs (diazepam, buspirone) showed no alteration (Graeff et al., 1993, 1998; Teixeira et al., 2000; Flausino et al., 2002; Beijamini and Andreatini, 2003; Poltronieri et al., 2003; Bakker et al., 2005; Mitte et al., 2005). Thus, apparently, the model showed good pharmacological validity. However, few antipanic drugs have been tested in the elevated T-maze and all are presumed to act by serotonergic action. Antipanic drugs with other mechanisms of action have not been studied and, thus, the ability of the elevated T-maze to detect non-serotonergic antipanic drugs remains unevaluated. This subject is important when we use the model to research new antipanic drugs, which may possess a non-serotonergic mechanism.

In order to extend the pharmacological validation of the elevated T-maze, the aim of the present study was to evaluate the effect of sodium valproate in rats submitted to the elevated T-maze. From the literature reviewed above, it was predicted that sodium valproate treatment would impair inhibitory avoidance acquisition (anxiolytic-like effect) and would increase latency for one-way escape (antipanic-like effect). Additionally, the study tested whether the effects of valproate were mediated by GABA type A receptors, by pre-treatment with picrotoxin.

2. Materials and methods

2.1. Animals

The subjects were adult male albino Wistar rats (250–320 g) from our own breeding colonies. They were housed in groups of five in polypropylene cages (cage size: 41 × 32 × 16.5 cm) with wood shavings as bedding under controlled conditions of light (12 h light–dark cycle, light on at 7:00 a.m.) and temperature (22 + 1 °C). Tap water and food pellets were available ad libitum throughout the experiment.

2.2. Drugs

Sodium valproate (300 mg/kg; Sanofi-Synthelabo) and picrotoxin (0.5 mg/kg; Sigma) were dissolved in distilled water (which was used as control solution) and administered intraperitoneally in a constant volume of 1.0 ml/kg 30 and 35 min (valproate and picrotoxin, respectively) prior to behavioral testing. Control animals received two vehicle injections in the same schedule. Four groups were formed ($n = 10$/group): saline–saline (sal sal), saline–valproate (sal val); picrotoxin–saline (pic sal); picrotoxin–valproate (pic val). The rats were tested in an order counter-balanced by treatment condition. The dose of both drugs was chosen based on data from the literature (studies cited in introduction) and pilot experiments in our laboratory that showed that a higher dose of sodium valproate (400 mg/kg) alone impairs motor coordination, while a higher dose of picrotoxin (1.0 mg/kg) alone increases inhibitory avoidance.

2.3. Apparatus

The elevated T-maze was made of black painted wood and had three arms of equal dimensions (50 × 10 cm). One arm, enclosed by 40 cm high walls, was perpendicular to two opposed open arms. To prevent the rats from falling off, the open arms were surrounded by a wood rim 1 cm high. The whole apparatus was elevated 50 cm above the floor. The experiments were performed with an observer inside the experimental room. The level of illumination was 40 and 80 lux on the floor of the closed arms and open arms, respectively.

2.4. Procedure

All evaluations were performed blind to the drug treatment and each model was always carried out by the same researcher. The intra-rater reliability (Intraclass Coefficient Correlation) of the scoring methods was between 0.90 and 0.97.

2.4.1. Inhibitory avoidance

A rat was placed at the end of the enclosed arm of the maze and the latency taken to withdraw from this arm was recorded in 3 successive trials. The first trial is designed as basal latency and represents the learning of the inhibitory avoidance behavior. The interval between trials was 30 s, during which the rat was placed in a polypropylene box identical to its home cage. Baseline latency (time to first withdrawal from the closed arm) was used as a secondary index of locomotor activity (Teixeira et al., 2000).

2.4.2. One-way escape

Thirty seconds after the last trial of inhibitory avoidance, the rat was placed at the end of one open arm and the latency to enter the enclosed arm (one-way escape) was recorded in 3 successive trials.

The rats were pre-exposed to one open arm during 30 min 24 h before the test (Teixeira et al., 2000; Flausino et al., 2002; Poltronieri et al., 2003). The cut off time for inhibitory avoidance and one-way escape was 300 s. After each trial the maze was cleaned with ethanol (10% v/v) to avoid possible bias due to odors and/or residues left by rats tested earlier.

2.4.3. Open-field test

Another group of rats were treated to the same schedules that were used in the elevated T-maze and tested in the open-field. The open-field apparatus is a square arena (50 × 40 × 63 cm), divided into 20 small units. The level of illumination on the floor of the open-field was 290 lux. The rats were placed individually in the center of the open-field and their locomotor activity (number of

units crossed) was recorded for 5 min. The open-field was washed with a water–alcohol (10%) solution before behavioral test of each rat, again to avoid possible bias due to odors and/or residues left by rats tested earlier. The number of units crossed in the open-field was taken as a primary index of locomotor activity.

2.5. Statistical analysis

Since the data from the elevated T-maze did not show homoscedasticity, they were analyzed by non-parametric tests: Kruskal–Wallis Analysis of Variance (ANOVA) followed by Dunn's Multiple Comparison test when appropriate. Data from the open-field test meet the parametric assumptions and thus they were analyzed by two-way ANOVA (factor 1, picrotoxin vs. saline; and factor 2, valproate vs. saline). The level of significance was $p<0.05$. The software GraphPad Prism 3.0 (GraphPad Software Inc., 1999) was used.

2.6. Ethics

All procedures were carried out in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Committee to Revise the Guide for the Care and Use of Laboratory Animals, 1996).

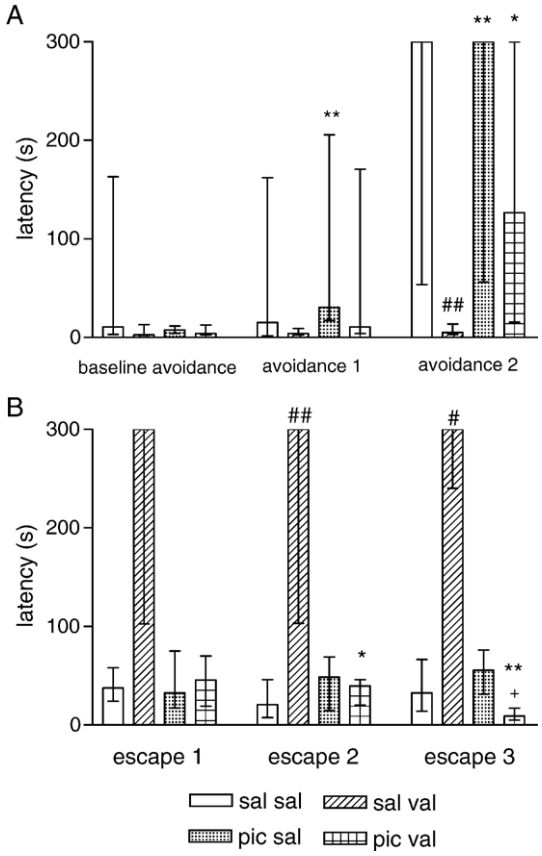

Fig. 1. The effects of acute administration of sodium valproate (300 mg/kg) and picrotoxin (0.5 mg/kg), alone or in combination, on the behavior of rats submitted to the elevated T-maze. Groups — sal sal: saline–saline; pic sal: picrotoxin–saline; sal val: saline–valproate; pic val: picrotoxin–valproate. Data represent median ± interquartile range ($n=10$/per group). A: inhibitory avoidance; B: one-way escape. $^{\#}p<0.05$ compared to saline–saline. $^{\#\#}p<0.01$ compared to saline–saline. $^{*}p<0.05$ compared to saline–valproate. $^{**}p<0.01$ compared to saline–valproate. $^{+}p<0.05$ compared to picrotoxin–saline.

3. Results

3.1. Inhibitory avoidance task (Fig. 1A)

There was no significant difference between groups in baseline latency ($H=5.439$, $p>0.10$). In contrast, there was a significant difference in avoidance 1 ($H=10.95$, $p<0.02$). The group treated with saline–valproate exhibited a lower latency than the picrotoxin–saline group. There was also a significant difference between groups in avoidance 2 ($H=18.49$, $p<0.001$). Post hoc analysis showed that saline–valproate demonstrated a lower latency than the saline–saline ($p<0.01$), picrotoxin–saline ($p<0.01$) and picrotoxin–valproate groups ($p<0.05$) (Fig. 1A).

3.2. Escape from open arms (Fig. 1B)

In escape 1, although Kruskal–Wallis ANOVA indicated a significant difference between treatments ($H=8.623$, $p<0.05$), post hoc analysis did not show any difference between the groups (all $p>0.05$).

In contrast, a significant difference between groups in escape 2 ($H=14.65$, $p<0.01$) and 3 ($H=25.99$, $p<0.0001$) was found. In escape 2, the saline–valproate group demonstrated a higher latency than the saline–saline group ($p<0.01$) and the picrotoxin–valproate group ($p<0.05$). In escape 3, the saline–valproate group demonstrated a higher latency than the saline–saline group ($p<0.05$) and the picrotoxin–valproate group ($p<0.01$). Additionally, this last group exhibited a lower latency than the picrotoxin–saline group ($p<0.05$).

3.3. Open-field

ANOVA found no significant effect for main effects of valproate [$F(1,36)=0.015$, $p>0.10$], of picrotoxin [$F(1,36)=1.267$, $p>0.10$], or interaction [$F(1,36)=3.657$, $p=0.06$]. The mean (±SEM) of each group was: saline–saline 63.2 (±4.7); saline–valproate = 77.0 (±7.6); picrotoxin–saline = 72.5 (±4.4); picrotoxin–valproate = 58.8 (±4.3).

4. Discussion

The results of the present study showed that sodium valproate impaired inhibitory avoidance and increased escape latency in the elevated T-maze, suggesting anxiolytic-like and panicolytic-like effects, respectively. The effect of sodium valproate on inhibitory avoidance is in agreement with data from other animal models of anxiety, which suggest an anxiolytic-like effect for this compound (Petersen and Lassen, 1981; Bovier et al., 1982; Myslobodsky et al., 1983; Shephard and Estall, 1984; Simiand et al., 1984; Vellucci and Webster, 1984; Corbett et al., 1991; de Angelis, 1995; Dalvi and Rodgers, 2001). The increase in time to escape from an open arm suggests a panicolytic-like effect for sodium valproate, which is in agreement with a valproate-

induced increase in escape latency and escape threshold in response to electrical stimulation of the dorsal periaqueductal gray — DPAG (Bovier et al., 1982), another animal model of panic disorder. These results were in agreement with clinical data indicating a putative panicolytic effect of sodium valproate (Primeau et al., 1990; Keck et al., 1993; Woodman and Noyes, 1994; Baetz and Bowen, 1998). Thus, these results support the proposed theory that the inhibitory avoidance task is related to generalized anxiety disorder and one-way escape is related to panic anxiety (Graeff et al., 1993, 1998; Teixeira et al., 2000; Poltronieri et al., 2003; Zanoveli et al., 2005).

Pre-treatment with picrotoxin, a noncompetitive GABA type A receptor antagonist, blocked both effects of valproate in the elevated T-maze. The picrotoxin effect on inhibitory avoidance is in agreement with previous studies that showed an antagonism of anxiolytic-like effect by picrotoxin pre-treatment (Liljequist and Engel, 1984; Vellucci and Webster, 1984; Shephard et al., 1985, 1990; Dalvi and Rodgers, 2001). Furthermore, similar to that was found by Dalvi and Rodgers (2001), in the present study the picrotoxin dose used was without intrinsic activity. These results strengthen the hypothesis of GABA type A receptors mediation of the anxiolytic-like effect of valproate. Besides this, the blockage of the valproate effect in the one-way escape indicates that the panicolytic-like effect of valproate is also mediated by GABA type A receptors. It is interesting to note that GABAergic drugs administered in DPAG impaired the response elicited by an electrical stimulation of this area (Brandão et al., 1982; Audi and Graeff, 1984) and impaired the one-way escape in the elevated T-maze (Bueno et al., 2005). These results suggest that DPAG may be the location of valproate panicolytic action, a hypothesis that should be confirmed in future studies.

It must be emphasized that all the effects observed in the elevated T-maze were probably not secondary to locomotor impairment, since no effect of the treatments was seen in the open-field. Additionally, an impairment of locomotor activity could account for impairment in latency in entering the open arms, increasing the inhibitory latency, but, in fact, a decrease in inhibitory avoidance was seen. Supporting this conclusion, no significant difference was seen in the baseline avoidance, a secondary measure of locomotor activity. The co-administration of valproate and picrotoxin did not change locomotor activity in the open-field test. These data agree with results found with conditioned suppression and Geller–Seifter tests where this association did not alter the unpunished behavior in rats (Vellucci and Webster, 1984; Shephard et al., 1990). In this line, picrotoxin did not alter the depressant effect of sodium valproate (400 mg/kg) in rat locomotor activity (Liljequist and Engel, 1984). In contrast, these results and those of the present study disagree with the data of Dalvi and Rodgers (2001), who found that valproate–picrotoxin co-administration increased the locomotor activity of mice in the elevated plus-maze. Although Dalvi and Rodgers suggested an interesting hypothesis for this unexpected result, that picrotoxin acted as partial antagonist of the anxiolytic effect of valproate, inducing an increase in locomotor activity, these effects could be also due to methodological differences among these studies (species; models). Aside from this discrepancy, the data found here indicated that the results found in the elevated T-maze were not due to a locomotor effect.

In summary, the present study shows that sodium valproate treatment exerts anxiolytic-like and panicolytic-like effects in the elevated T-maze, which indicated that the elevated T-maze is sensitive to panicolytic compounds that act through non-serotonergic mechanisms. Moreover, since these effects of valproate were blocked by pre-treatment with picrotoxin, this suggests that they were mediated by GABA type A receptors. In addition, these results provide additional data for the predictive validity of the elevated T-maze and reinforce its utility in the search for new anxiolytic and panicolytic drugs.

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