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Proconvulsive effect of the $GABA_B$ receptor antagonist, SCH 50911, in rats undergoing ethanol withdrawal syndrome

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Abstract

The present study investigated the effect of the $GABA_B$ receptor antagonist, SCH 50911 [(2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid], on the occurrence of seizures in ethanol-dependent rats undergoing ethanol withdrawal syndrome. The acute administration of nonconvulsive doses of SCH 50911 (0, 100, 170 and 300 mg/kg, i.p.) resulted in a dramatic facilitation of spontaneous seizure occurrence. This finding, together with the reported ability of the $GABA_B$ receptor agonist, baclofen, to suppress seizures associated to ethanol withdrawal syndrome, suggests that the $GABA_B$ receptor may be part of the neural substrate underlying the hyperexcitability of ethanol withdrawal syndrome.

Keywords: $GABA_B$ receptor antagonist, SCH 50911; Seizures; Ethanol withdrawal syndrome; (Rat)

1. Introduction

Accumulating evidence suggests that the pharmacological stimulation of the $GABA_B$ receptor may inhibit the intensity of withdrawal syndrome of some drugs of abuse, including morphine (Zarrindast and Mausa-Ahmadi, 1999; Akhondzadeh et al., 2000; Bexis et al., 2001; Diaz et al., 2001; Kemmling et al., 2002) and barbiturates (Benedito and Leite, 1981). Further, administration of nonsedative doses of the $GABA_B$ receptor agonist, baclofen, has been found to protect from audiogenic seizures associated to ethanol withdrawal syndrome and reverse other withdrawal signs, including tremors and anxiety-like behaviors, in rats and mice rendered physically dependent on ethanol (File et al., 1991; Colombo et al., 2000; however, see Humeniuk et al., 1994; Mead and Little, 1995). More recently, the acute administration of a relatively low dose of baclofen has been found to result in the rapid disappearance of ethanol withdrawal symptomatology in human alcoholics (Addolorato et al., 2002). Taken together, the above results suggest the involvement of the $GABA_B$ receptor in the neural substrate underlying ethanol withdrawal syndrome.

To our knowledge, only a few studies have extended the investigation on the pharmacological manipulation of ethanol withdrawal syndrome by $GABA_B$ agents to the antagonists. Specifically, Mead and Little (1995) reported that the $GABA_B$ receptor antagonist, CGP 35348 [(3-aminopropyl) (diethoxymethyl) phosphinic acid], decreased hyperexcitability (measured as responses to handling) during ethanol withdrawal in mice; however, CGP 35348 produced a similar effect in control mice which had not been treated with ethanol, posing some question on the specificity of the drug action. Humeniuk et al. (1994) found that CGP 35348 and the other $GABA_B$ receptor antagonist, β-phenyl-β-alanine, reduced, to a very limited extent, the intensity of tremors associated to ethanol withdrawal in mice. To further evaluate this issue, the present study assessed the effect of the $GABA_B$ receptor antagonist, SCH 50911 [(2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid], on the occurrence of seizures in rats made physically dependent on ethanol by

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the repeated administration of intoxicating doses of ethanol and showing clear signs of ethanol withdrawal syndrome at the time of SCH 50911 administration.

2. Materials and methods

2.1. Animals

Male Wistar rats (Harlan Nossan, Correzzana, MI, Italy), weighing 275–300 g at the start of the experiment, were used. After delivery to our facilities, rats were left undisturbed for 7 days to acclimatize to new housing conditions. Animals were housed five per cage with wood chip bedding under an artificial light–dark cycle of 12:12 h (lights on at 7:00), at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%. Rats were given free access to water and standard laboratory food (MIL Morini, San Polo d'Enza, RE, Italy) throughout the entire experimental period.

2.2. Intoxication procedure

Rats were rendered physically dependent on ethanol by the method of Majchrowicz (1975). This consisted of four daily administrations of ethanol solution (20% w/v, in tap water) by intragastric gavage for six consecutive days, in order to maintain constant blood ethanol concentrations. Animals were treated at 6:00, 12:00, 18:00 and 24:00. Initially, 4 g/kg ethanol were given to all rats. The assessment of subsequent doses was determined individually for each rat at the above administration times on the basis of the observed degree of intoxication using the intoxication–dose relationship conceived by Majchrowicz (1975). Six successive stages of intoxication were defined: neutrality, sedation, ataxia 1, 2 and 3, loss of righting reflex. Ethanol doses, ranging from 0 to 5 g/kg, were inversely related to the degree of intoxication. The degree of intoxication was assessed and the ethanol dose was chosen by operators trained in use of the same evaluation criteria.

Rats were weighed once a day (at 9:00). During chronic ethanol treatment, rats spent most of the time in a severe state of intoxication, unable to eat by themselves. Therefore, the loss in body weight was partially compensated by the daily oral administration (at 9:00) of 20 g/kg liquid diet (Isomil, M&R, Zwolle, The Netherlands).

2.3. Withdrawal assessment

About 15 h after the last ethanol administration, intensity of ethanol withdrawal signs was evaluated in each rat scoring 10 separate items. A four-point scale (from 0 to 3, paralleling increased frequency of occurrence and degree of severity of items) modified from a scale described by Lal et al. (1988) was used. Some items (shakes, jerks, head tremors, bracing posture and spontaneous convulsions) were rated before touching the rat, while assessment of other items required palpation (namely, general tremors, tail tremors, rigidity of muscle tone and tail rigidity) or handling stimulation (vocalization). The sum of the 10 values was the total score assigned to each rat on each observation. Scores of 7–8 indicated a neutrality state, corresponding to healthy and undrugged rats.

2.4. Drug treatment and seizure assessment

Immediately after evaluation of alcohol withdrawal score, rats were divided into four groups ($n = 9$), matched for withdrawal score. Rats which convulsed prior to SCH 50911 administration were excluded from the study. SCH 50911 (synthesized by G.M. as previously described by Blythin et al., 1996) was dissolved in saline and injected i.p. at the doses of 0, 100, 170 and 300 mg/kg (injection volume: 2 ml/kg). SCH 50911 doses were chosen on the basis of preliminary experiments which demonstrated that the seizure score (see above) elicited by these doses of SCH 50911 in healthy, undrugged adult male Wistar rats was equal to 0.

Immediately after SCH 50911 injection, rats were placed in single cages, located in a quiet soundproof room, and observed for 75 min. Seizure severity was assessed by means of the seven-point scale introduced by Follesa et al. (1999). Briefly, score 0 was assigned to the lack of any response, score 1 to the occurrence of ear and facial twitching, score 2 to 1–20 myoclonic body jerks in 10-min intervals, score 3 to more than 20 myoclonic body jerks in 10-min intervals, score 4 to clonic forelimb convulsions, score 5 to generalized clonic convulsions with episodes of rearing and falling down and score 6 to generalized convulsions with episodes of tonic extension and status epilepticus. The final score of each rat was the maximum value assigned during the observation interval. Seizure score was assessed by operators trained in use of the same evaluation criteria and blind as to group allocation.

2.5. Statistical analysis

Statistical evaluation of ethanol withdrawal score and seizure score was performed by the one-way analysis of variance (ANOVA), followed by the Newman–Keuls test for post hoc comparisons.

3. Results

Rats from the different experimental groups did not differ in intensity of ethanol withdrawal syndrome at the time of SCH 50911 injection. Indeed, the average withdrawal score was 15.1 ± 0.6, 15.1 ± 0.4, 15.1 ± 0.5 and 15.0 ± 0.5 in the rat groups assigned to 0, 100, 170 and 300 mg/kg SCH 50911 treatment, respectively ($F(3,32) = 0.01$, $P > 0.05$).

Administration of SCH 50911 resulted in a significant, dose-dependent increase in seizure score ($F(3,32) = 9.57$, $P < 0.0005$). Post hoc analysis revealed that all doses of SCH

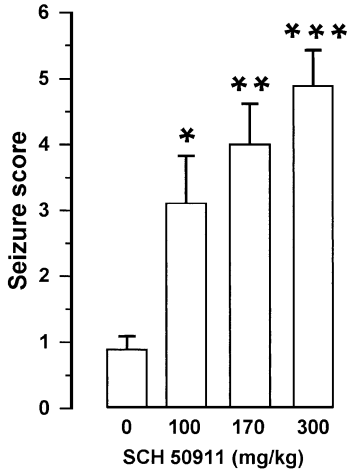

Fig. 1. Potentiation of seizure score after acute administration of the $GABA_B$ receptor antagonist, SCH 50911, in rats undergoing ethanol withdrawal syndrome. Rats were rendered physically dependent on ethanol by the repeated administration of intoxicating amounts of ethanol. SCH 50911 (0, 100, 170 and 300 mg/kg) was injected i.p. 15 h after the last ethanol administration, i.e. when ethanol withdrawal signs had reached their maximal intensity. Rats were observed for 75 min after SCH 50911 administration. Seizure score was the maximal value reached by each rat on a seven-point scale. Each bar is the mean ± S.E.M. on $n = 9$ rats. *: $P < 0.05$, **: $P < 0.005$ and ***: $P < 0.0005$ in comparison to saline-treated rats (Newman–Keuls test).

50911 produced seizure scores significantly higher than that assessed in saline-treated rats (Fig. 1). In the 300-mg/kg SCH 50911-dosed group, four out of nine rats displayed repeated episodes of tonic-clonic seizures (scoring 6).

4. Discussion

The results of the present study indicate that the acute administration of nonconvulsive doses of the $GABA_B$ receptor antagonist, SCH 50911, facilitated the onset of seizures associated with ethanol withdrawal syndrome in rats. These results are complementary with the recently reported ability of the $GABA_B$ receptor agonist, baclofen, to suppress both spontaneous and audiogenic seizures in rats undergoing ethanol withdrawal syndrome (Colombo et al., 2000). Taken together, these results add further support to the hypothesis that the $GABA_B$ receptor may be part of the neural substrate underlying the hyperexcitability of ethanol withdrawal syndrome.

A complex relationship appears to exist between $GABA_B$ receptors and seizures. Administration of high doses of the $GABA_B$ receptor antagonists, CGP 36742 (3-aminopropyl-*n*-butyl-phosphinic acid) and CGP 56999 ([3-[1-(*R*)-[(3-cyclohexylmethyl)hydroxyphosphinyl]-2-(*S*)-hydroxy-propyl]amino]ethyl]-benzoic acid), has been found to produce convulsive seizures in nonepileptic rats (Badran et al., 1997; Vergnes et al., 1997) and promote, at a lower dose range, the occurrence of sound-induced seizures in rats sensitive to audiogenic seizures (Vergnes et al., 1997). Further, CGP 56999 (Badran et al., 1997) and CGP 35348 (Veliskova et al., 1996) had proconvulsive activity in a pentylenetetrazole-induced model of tonic-clonic seizures in rats and mice. Conversely, baclofen had a protective effect on seizures produced by pentylenetetrazole (Veliskova et al., 1996) and kainic acid (Ault et al., 1986) in rats and reduced the electrophysiological correlates of epilepsy in rat hippocampal slices (Ault and Nadler, 1983; Morrisett et al., 1993). In contrast, accumulating lines of experimental evidence indicate that different $GABA_B$ receptor antagonists, including SCH 50911, inhibited (Liu et al., 1992; Hosford et al., 1995; Snead, 1996; Aizawa et al., 1997), while baclofen provoked (Liu et al., 1992; Snead, 1996), absence seizures in different animal models of absence epilepsy.

The protective effect of $GABA_B$ receptor antagonists in models of generalized absence seizures and their proconvulsive activity in other experimental models of seizures, including ethanol withdrawal syndrome, as well as the opposite effects of baclofen, suggest that different cellular mechanisms and/or brain structures may be involved in these phenomena. Accordingly, a dysfunction in $GABA_B$ receptor-mediated transmission within thalamocortical circuits has been proposed to be responsible for the onset and maintenance of absence seizures (see Caddick and Hosford, 1996; Futatsugi and Riviello, 1998). In contrast, the $GABA_B$ receptor-mediated modulation of glutamate neurotransmission through the *N*-methyl-D-aspartate (NMDA) receptor might be the substrate of the effects of $GABA_B$ receptor agents on ethanol withdrawal syndrome. Indeed, it has been repeatedly suggested that ethanol withdrawal hyperexcitability is associated with an increased function of the NMDA subtype of glutamate receptor in hippocampus and possibly other brain regions; for instance, (a) NMDA receptors are "up-regulated" in the rodent hippocampus when manifestations of ethanol withdrawal syndrome occur (e.g. Grant et al., 1990, 1992; Valverius et al., 1990; Gulya et al., 1991; Iorio et al., 1992; Morgan et al., 1992; Sanna et al., 1993; Snell et al., 1993, 1996; Trevisan et al., 1994; Hu et al., 1996) and (b) administration of NMDA receptor antagonists reduced the intensity of seizures and other signs of ethanol withdrawal syndrome (e.g. Grant et al., 1990, 1992; Morrisett et al., 1990; Liljequist, 1991; Erden et al., 1999; Gatch et al., 1999; Bienkowski et al., 2001). It has been demonstrated that $GABA_B$ receptors modulate NMDA-mediated responses in the rat hippocampus (Morrisett et al., 1991). Further, a recent electrophysiological study demonstrated that baclofen mimicked ethanol-induced inhibition of NMDA-activated hippocampal neurons and that the effect of both baclofen and ethanol was blocked by CGP 35348 (Steffensen et al., 2000). Therefore, baclofen-induced activation of $GABA_B$ receptors might counterbalance the enhanced function of glutamate excitatory neurotransmission associated to ethanol withdrawal syndrome, resulting in attenuation of the withdrawal symptomatology (File et al., 1991; Colombo et al., 2000; Addolorato et al., 2002; however, see Humeniuk et al., 1994; Mead and Little, 1995); conversely, the proconvulsive

activity of SCH 50911 observed in the present study might be the result of the removal of an inhibitory tone on an already stimulated NMDA neurotransmission.

In conclusion, the results of the present study suggest that the pharmacological blockade of the $GABA_B$ receptor may promote the occurrence of seizures associated to ethanol withdrawal syndrome in rats physically dependent on ethanol and that the $GABA_B$ receptor may be part of the neural substrate mediating the hyperexcitability associated to ethanol withdrawal syndrome.

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