ELSEVIER

European Journal of Pharmacology 314 (1996) 277–280

ejp

Short communication

Competitive NMDA receptor antagonist, CGP 40116, substitutes for the discriminative stimulus effects of ethanol

Przemyslaw Bienkowski [a,b], Roman Stefanski [a], Wojciech Kostowski [a,b,*]

[a] *Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Sobieskiego 1/9, PL-02957, Warsaw, Poland*

[b] *Department of Experimental and Clinical Pharmacology, Medical High School, Warsaw, Poland*

Received 28 May 1996; revised 13 August 1996; accepted 16 August 1996

Abstract

A drug discrimination procedure was used to compare the ability of competitive (CGP 37849, D,L-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoate; CGP 40116, D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoate) and non-competitive (dizocilpine) NMDA receptor antagonists to substitute for ethanol in rats trained to discriminate between a 1.0 g/kg dose of ethanol (i.p.) and saline. Dizocilpine (0.1–0.3 mg/kg) substituted partially for ethanol at doses that markedly reduced the rate of responding. CGP 37849 (1.25–5.0 mg/kg) substituted partially for ethanol and suppressed the response rate. CGP 40116 (0.5–2.5 mg/kg), an active D-stereoisomer of CGP 37849, completely substituted (88%) for ethanol, and caused only moderate suppression of the response rate.

Keywords: Ethanol; NMDA receptor antagonist; Drug discrimination; (Rat)

1. Introduction

Ethanol has been found to antagonise NMDA receptor-mediated biochemical and electrophysiological responses, leading to the hypothesis that at least certain central effects of ethanol may result from its interaction with the NMDA receptor complex (Lovinger et al., 1989; Hoffman et al., 1989; Simson et al., 1991). In line with this hypothesis are data showing that NMDA receptors may contribute to ethanol withdrawal and intoxication (Danysz et al., 1992; Sanna et al., 1993). Furthermore, using a drug discrimination procedure it has been reported that the discriminative stimulus effects of ethanol are mediated, at least in part, by antagonism of NMDA receptor conductance, as evidenced by the ability of NMDA receptor antagonists to substitute for ethanol in pigeons (Grant et al., 1991b) and rats (Sanger, 1993). In rats both non-competitive and competitive NMDA receptor antagonists have been reported to produce either full or partial substitution for ethanol (Sanger, 1993; Shelton and Balster, 1994; Koek et al., 1995) and the generalisation was usually accompanied by a substantial reduction in the response rate. Evidence exists suggesting that the degree of substitution for ethanol produced by competitive and non-competitive NMDA receptor antagonists may depend upon the training dose of ethanol used (Grant and Colombo, 1993).

To further examine the ability of NMDA receptor antagonists to substitute for the ethanol interoceptive cue in rats, we compared the effects of two competitive NMDA receptor antagonists, CGP 37849 (D,L-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoate) and its active D-stereoisomer, CGP 40116 (D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoate) (Fagg et al., 1990; Schmutz et al., 1991), with those of a non-competitive NMDA receptor antagonist, dizocilpine, in rats trained to discriminate between 1.0 g/kg ethanol and saline.

2. Materials and methods

2.1. Subjects

Male Wistar rats (300–330 g at the beginning of the study) were used. The rats were individually housed in

* Corresponding author at address a. Tel./Fax: (48-22) 427-644; Fax: (48-22) 642-5357.

PII S0014-2999(96)00658-9

standard plastic cages, in a temperature-controlled (21 ± 1°C) vivarium with 12-h light-dark cycle (lights on at 7 a.m.). Rats were maintained at 80–85% of their free-feeding body weight by restriction of their daily food rations. Tap water was available ad libitum.

2.2. Experimental procedure

The animals were trained to press either right or left levers for sweetened milk reinforcement in standard operant cages (Coulbourn Instruments, Allentown, PA, USA) enclosed within sound attenuating and ventilated cubicles (six rats were previously used for substitution and antagonism tests with 5-HT_3 receptor ligands, and six rats were completely naive). Discrimination training commenced once lever pressing on either right or left levers was established on a fixed ratio, FR10, schedule of reinforcement. Fifteen minutes before daily (Monday–Friday) 15-min test sessions, the rats were injected i.p. with ethanol (1.0 g/kg, 10% v/v; 13 ml/kg) and were required to press one of the levers (ethanol-appropriate lever) to receive reinforcement. After i.p. injection of saline (0.9% NaCl) the rats were required to press the opposite lever (saline-appropriate lever). Ethanol or saline was administered according to two alternating sequences: ethanol, saline, saline, ethanol, ethanol or saline, ethanol, ethanol, saline, saline. Lever selection was considered correct if the rat made ≤ 2 responses on the inappropriate lever before completing the first FR10 on the appropriate lever.

Substitution and dose-response tests (1–2/week) were carried out when the rats had achieved the discrimination criteria of ≤ 2 incorrect responses before completing the first FR10 and a response rate ≥ 0.45/s, in nine out of ten sessions. During the substitution and dose-response test sessions, the lever on which the first ten responses occurred continued to be reinforced for the remainder of the 15-min session. Responses on the other lever were recorded but not reinforced. Only rats that met the discrimination criteria during at least five consecutive training sessions were used in subsequent test sessions.

During the dose-response tests the rats received different doses of ethanol (0.25, 0.5, 0.75 and 1.0 g/kg, 10% v/v) 15 min before the start of the test. Dizocilpine (0.1–0.3 mg/kg; (+)-MK 801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptan-5,10-imine, RBI, Natick, MA, USA) was given i.p. 15 min before the test. CGP 37849 (1.25–5.0 mg/kg) and CGP 40116 (0.5–2.5 mg/kg; Ciba-Geigy, Switzerland) were administered i.p. 60 min before the test. An injection volume of 13 ml/kg was used for all drugs except for the lower ethanol doses.

2.3. Statistics

Data were expressed as a percentage (±S.E.M.) of ethanol-appropriate responding prior to the completion of the first FR10. The operational definition of complete stimulus substitution was 80% (or more) of responding on the ethanol-appropriate lever (Grant and Colombo, 1993).

The response rate was calculated by dividing the total number of responses by the total session time in seconds. ED_{50} values were determined using the method of Litchfield and Wilcoxon (Tallarida and Murray, 1987). Student's *t*-test two-tailed was used to determine the statistical significance of differences between response rates.

3. Results

The acquisition of ethanol discrimination required an average of 57 training sessions. The ED_{50}, calculated on the basis of the dose-response tests, was 0.51 g/kg (C.L. 0.35–0.74 g/kg). The training dose of ethanol did not

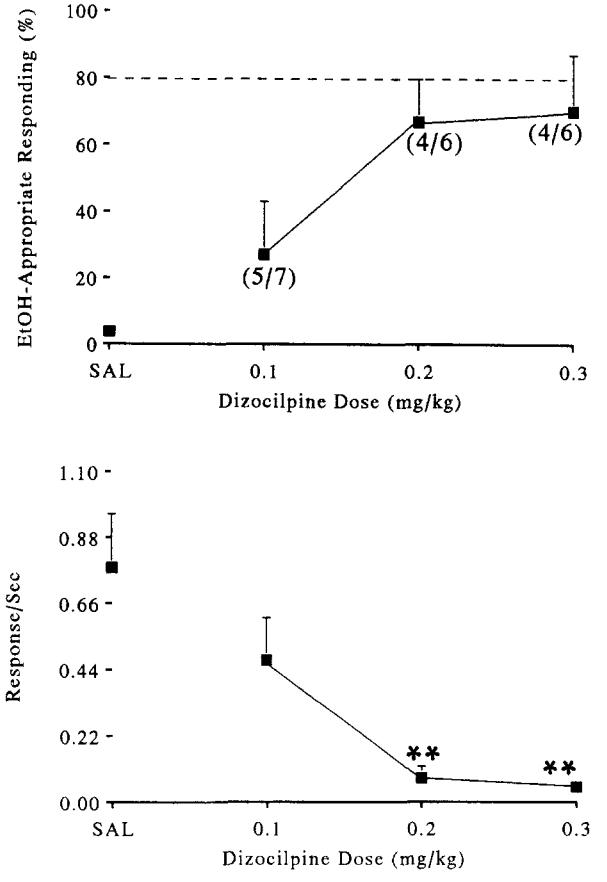

Fig. 1. Mean (±S.E.M.) percentage of ethanol-appropriate responding prior to the completion of the first reinforcement (upper panel) and mean (±S.E.M.) response rate (lower panel) following different doses of dizocilpine in rats trained to discriminate 1.0 g/kg of ethanol from saline. ** $P < 0.01$. The number of rats completing the test session and the number of rats tested in each session are shown as a ratio adjacent to each of the points. Horizontal line represents the operational definition of complete stimulus substitution.

produce any significant effect on the mean rate of responding.

Dizocilpine substituted partially for ethanol but only at doses (0.2–0.3 mg/kg) causing marked depression of the response rate. The maximum level of ethanol-appropriate responding (70.7%) was observed at the 0.3 mg/kg dose in four out of six animals that were still able to respond (Fig. 1, upper panel). Three out of four rats responding after 0.3 mg/kg of dizocilpine completed only one FR10 (i.e. obtained only one reinforcement).

CGP 37849 substituted partially (68%) for ethanol at the dose of 5.0 mg/kg, and reduced the response rate as well (Fig. 2, left panel). CGP 40116 produced dose-dependent ethanol-appropriate responding. Partial substitution (66.6%) occurred at the dose of 1.0 mg/kg and (in contrast to dizocilpine and CGP 37849) was not associated with a reduction of the response rate. Full substitution (88%) for ethanol was observed at the dose of 2.5 mg/kg. This dose was able to decrease the mean response rate to 43% of the saline control value (Fig. 2, right panel).

4. Discussion

Our results are consistent with those of previous drug discrimination studies showing that both competitive and non-competitive NMDA receptor antagonists are capable of substituting for ethanol in ethanol-trained rats (Grant and Colombo, 1993; Sanger, 1993; Shelton and Balster, 1994). Some of these studies reported that the non-competitive NMDA antagonist dizocilpine substituted completely for ethanol while in our hands this compound substituted for ethanol only partially. Procedural differences such as route of administration and dose of ethanol tested and small number of animals used may explain this discrepancy. Grant and Colombo (1993) reported that dizocilpine

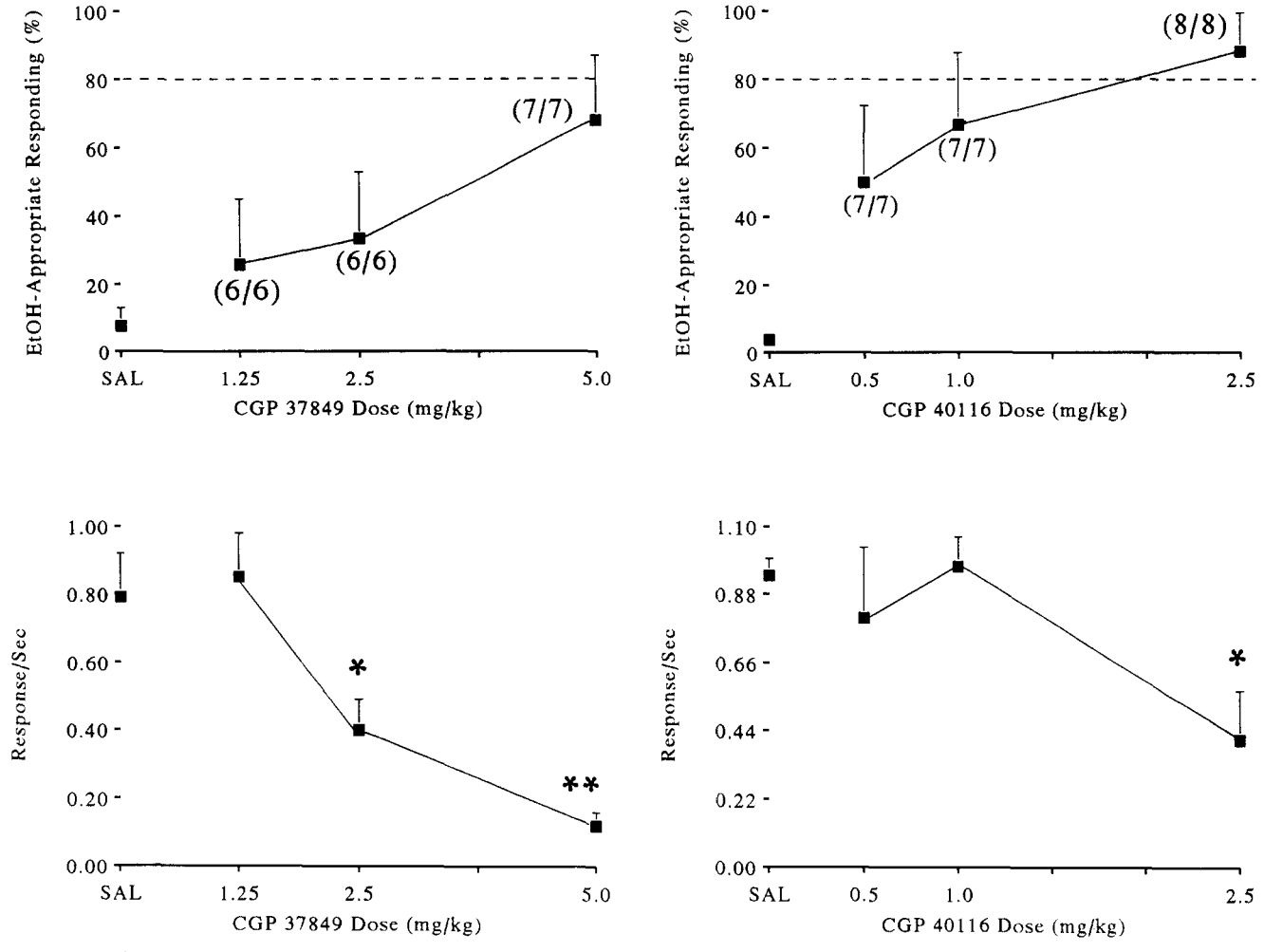

Fig. 2. Mean (±S.E.M.) percentage of ethanol-appropriate responding prior to the completion of the first reinforcement (upper panel) and mean (±S.E.M.) response rate (lower panel) following different doses of CGP 37849 and CGP 40116 in rats trained to discriminate 1.0 g/kg of ethanol from saline. * $P < 0.05$, ** $P < 0.01$. The number of rats completing the test session and the number of rats tested in each session are shown as a ratio adjacent to each of the points. The horizontal line represents the operational definition of complete stimulus substitution.

fully substituted for intragastrically administered higher doses of ethanol (1.5–2.0 g/kg) but only partial substitution occurred in the 1.0 g/kg ethanol-trained rats. Also, only partial substitution of dizocilpine and phencyclidine for ethanol (1.25 g/kg, i.p.) was recently reported by Koek et al. (1995). Notably, Shelton and Balster (1994), using rats trained to discriminate 1.0 g/kg ethanol (i.p.) from saline, showed that dizocilpine (0.2 mg/kg) substituted fully for ethanol, but only in two out of seven rats that were still able to respond.

The most important finding of our study is that the competitive NMDA receptor antagonist CGP 40116 fully substitutes (according to the operational definition) for 1.0 g/kg ethanol at a dose which, contrary to dizocilpine and CGP 37849, produced only a partial reduction of the response rate. This result is in line with other reports showing that other competitive NMDA receptor antagonists, CPPene and CGS 19755, fully substitute for intragastrically (Grant et al., 1991a; Grant and Colombo, 1993) or i.p. administered ethanol (1.0 g/kg) (Sanger, 1993). Furthermore, CGP 40116 (D-stereoisomer) seems to be at least 8-fold more potent than CGP 37849 (racemate) to produce ethanol-like discriminative stimulus effects, but the potency to reduce the response rate was similar for the two compounds (Fig. 2). In our present study, however, CGP 40116 was administered in a relatively narrow dose range and future experiments with lower doses of the compound would be helpful to arrive at definitive conclusions. Another important point is to test the inactive L-stereoisomer CGP 40117 (Fagg et al., 1990; Schmutz et al., 1991) under the same experimental conditions.

In conclusion, these data show that CGP 40116 substituted for ethanol and encourage the search for compounds that interact with the NMDA receptor complex and modify the centrally mediated subjective effects of ethanol. It would be of special interest to test if compounds with agonistic properties against the NMDA receptor complex antagonise the discriminative stimulus effects of ethanol (or at least ethanol-appropriate responding induced by NMDA receptor antagonists).

Acknowledgements

This study was supported by The State Agency for Prevention of Alcohol Related Problems, Warsaw, Poland. The authors thank Ciba-Geigy AG, Pharma Research (Basel, Switzerland) for its gift of CGP 40116 and CGP 37849.

References

Danysz, W., W. Dyr, E. Jankowska, S. Glazewski and W. Kostowski, 1992, The involvement of NMDA receptors in acute and chronic effects of ethanol, Alcohol. Clin. Exp. Res. 16, 499.

Fagg, G.E., H.R. Olpe, M.F. Pozza, J. Band, M. Steinmann, M. Schitz, C. Portet, P. Baumann, K. Thedinga, H. Bittiger, H. Allgeier, R. Heckendorn, C. Angst, D. Brundish and J.G. Dingwall, 1990, CGP 37849 and CGP 39551: novel and potent competitive *N*-methyl-D-aspartate receptors antagonists with oral activity, Br. J. Pharmacol. 99, 791.

Grant, K.A. and G. Colombo, 1993, Discriminative stimulus effects of ethanol: effect of training dose on the substitution of *N*-methyl-D-aspartate antagonists, J. Pharmacol. Exp. Ther. 264, 1214.

Grant, KA., G. Colombo and B. Tabakoff, 1991a, Competitive and non-competitive antagonists of the NMDA receptor complex have ethanol-like discriminative stimulus effects in rats, Alcohol. Clin. Exp. Res. 15, 321.

Grant, K.A., J.S. Knisely, B. Tabakoff, J.E. Barrett and R.L. Balster, 1991b, Ethanol-like discriminative stimulus effects of non-competitive *N*-methyl-D-aspartate antagonists, Behav. Pharmacol. 2, 87.

Hoffman, P.L., C.S. Rabe, F. Moses and B. Tabakoff, 1989, *N*-Methyl-D-aspartate receptors and ethanol: inhibition of calcium flux and cyclic GMP production, J. Neurochem. 52, 1937.

Koek, W., M.S. Kleven and F.C. Colpaert, 1995, Effects of the NMDA antagonist, dizocilpine, in various drug discriminations: characterization of intermediate levels of drug lever selection, Behav. Pharmacol. 6, 590.

Lovinger, D.M., G. White and F. Weight, 1989, Ethanol inhibits NMDA-activated ion current in hippocampal neurones, Science 243, 1721.

Sanger, D.J., 1993, Substitution by NMDA antagonists and other drugs in rats trained to discriminate ethanol, Behav. Pharmacol. 4, 523.

Sanna, E., M. Serra, A. Cossu, G. Colombo, P. Follesa, T. Cuccheddu, A. Concas and G. Biggio, 1993, Chronic ethanol intoxication induces differential effects on $GABA_A$ and NMDA receptor function in the rat brain, Alcohol. Clin. Exp. Res. 17, 115.

Schmutz, M., A. Jeker, K. Klebs, C. Portet, M. Pozza, G.E. Fagg, R. Heckendorn and H. Allgeier, 1991, CGP 40116/CGP 43487: competitive *N*-methyl-D-aspartate receptor antagonists with potent oral anticonvulsant activity, Epilepsia 32 (Suppl. 1), 59.

Shelton, K.L. and R.L. Balster, 1994, Ethanol drug discrimination in rats: substitution with GABA agonists and NMDA antagonists, Behav. Pharmacol. 5, 441.

Simson, P.E., H.E. Criswell, K.B. Johnson, R.E. Hicks and G.R. Breese, 1991, Ethanol inhibits NMDA-evoked electrophysiological activity in vivo, J. Pharmacol. Exp. Ther. 257, 225.

Tallarida, R.J. and R.B. Murray, 1987, Manual of Pharmacological Calculations, 2nd edn. (Springer, New York, NY).