Pharmacology Biochemistry and Behavior, Vol. 58, No. 4, pp. 969–973, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00034-8

Discriminative Stimulus Properties of Ethanol in the Rat: Differential Effects of Selective and Nonselective Benzodiazepine Receptor Agonists

PRZEMYSLAW BIENKOWSKI,*† KRYSTYNA IWINSKA,† ROMAN STEFANSKI‡[1] AND WOJCIECH KOSTOWSKI*†[2]

*Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Al. Sobieskiego 1/9, PL-02957, Warsaw, Poland
†Department of Experimental and Clinical Pharmacology, Warsaw Medical University, P0-00927, Warsaw, Poland
‡Preclinical Pharmacology Laboratory, Behavioral Pharmacology and Genetic Section, National Institute on Drug Abuse, P.O. Box 5180, Baltimore, MD 21224, USA

Received 12 March 1996; Revised 21 February 1997; Accepted 21 February 1997

BIENKOWSKI, P., K. IWINSKA, R. STEFANSKI AND W. KOSTOWSKI. *Discriminative stimulus properties of ethanol in the rat: Differential effects of selective and nonselective benzodiazepine receptor agonists.* PHARMACOL BIOCHEM BEHAV **58**(4) 969–973, 1997.—Rats were trained to discriminate between ethanol (1.0 g/kg; 10% v/v) and saline under a fixed ratio 10 schedule of sweetened milk reinforcement. Both diazepam [nonselective, full benzodiazepine (BZ) receptors agonist] and bretazenil (nonselective, partial BZ receptor agonist) produced dose-dependent ethanol-appropriate responding (>75%). Neither diazepam nor bretazenil affected the response rate at the doses producing maximal generalisation from ethanol. In contrast, zolpidem (full BZ1 receptor agonist) and abecarnil (full BZ1/full or partial BZ2 receptor agonist) produced only moderate (<50%) ethanol-appropriate responding when tested up to doses that markedly decreased the overall response rate. These results suggest that: 1) there are no major differences between full and partial, nonselective BZ receptor agonists in their ability to substitute for 1.0 g/kg dose of ethanol; 2) stimulation of BZ1 receptors alone is not sufficient to produce ethanol-like discriminative stimulus effects in the rat. © 1997 Elsevier Science Inc.

Ethanol GABA$_A$ receptor complex Benzodiazepine receptor Drug discrimination Rat

THE GABA$_A$ receptor (the GABA-benzodiazepine (BZ) receptor/ionophore complex) has been identified as an important target for ethanol in the central nervous system [(13,15,23,26); for review, see (8)]. Several electrophysiological and biochemical studies, for example, have shown that ethanol enhances GABA-mediated inhibition of neuronal activity and increases GABA-induced chloride flux in many, although not all, brain areas (6,7,15,26). Interestingly, sensitivity of different regions of the rat brain to GABA-associated depressant effects of ethanol seems to be correlated with zolpidem binding (6,7). Zolpidem (imidazopyridine derivative) acts as a full BZ receptor agonist and shows high affinity for GABA$_A$ receptors containing an α_1 subunit (corresponding to the BZ1 receptor subtype) and considerably lower affinity for receptors containing α_2, α_3, or α_5 subunits (corresponding to the BZ2 receptor subtype) (6,7,16,20). Taken together, these re-

[1]Present address: Preclinical Pharmacology Laboratory, Behavioral Pharmacology and Genetic Section, National Institute on Drug Abuse, P.O. Box 5180, Baltimore, MD 21224.

[2]Requests for reprints should be addressed to Wojciech Kostowski, Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Al. Sobieskiego 1/9, PL-02957 Warsaw, Poland.

sults have led some authors to a hypothesis that the population of $GABA_A$ receptors with BZ1 receptor pharmacological characteristics may be particularly sensitive to ethanol (6,7).

It has been suggested that a drug discrimination procedure in animals is the closest available experimental model for assessing the potential of drugs to produce subjective effects in humans (4,10,18). This procedure is useful for identifying potential receptors that mediate the stimulus effects of drugs. In animals trained to discriminate a particular drug from vehicle, the ability of other drugs to substitute for the training drug, or to antagonize the effects of the training drug, can be tested (4,10,24). In agreement with the results mentioned above, many compounds enhancing the $GABA_A$ receptor function substitute for ethanol in the drug discrimination procedure (1,2,11). Thus, barbiturates, benzodiazepines, and certain neurosteroids with GABAergic properties, have consistently been reported to produce ethanol-like stimulus effects in different animal species discriminating ethanol from its vehicle (1,2,9,11,14,18,21,22).

The pharmacological characterization of the BZ receptor-positive modulators ranges from the full or partial nonselective agonists to the selective agonists acting on a certain BZ receptor subtype (e.g., zolpidem, see above) (5,12,16). Thus, diazepam behaves as a full agonist at $GABA_A$ receptors containing α_1, α_2, α_3, and α_5 subunits, while the partial nonselective agonist, bretazenil, displays considerably lower intrinsic activity at all of these receptors [for review, see (5,16,20)]. On the other hand, abecarnil (β-carboline derivative) acts as a full agonist at $GABA_A$ receptors containing an α_1 or α_3 subunit but as a partial agonist at receptors containing an α_2 or α_5 subunit (5,12,16). In addition, a relative selectivity towards the BZ1 receptor subtype has also been reported for this compound (12,16).

To confirm and extend the results of previous reports (14,18,21,22), we decided to compare the effects of different BZ receptor agonists (i.e., diazepam, bretazenil, abecarnil, and zolpidem) in rats trained to discriminate 1.0 g/kg ethanol from saline.

METHOD

Subjects

Male Wistar rats (300–330 g at the beginning of the study), were used. They were housed individually and maintained at ~80% of their free-feeding body weight by restricting daily food (Bacutil, Poland) to 15–20 g. Tap water was available ad lib. The animals were kept with standard laboratory conditions at 22 ± 1°C, 60% humidity, and 12-h light–dark cycle (lights on at 0700 h). All experimental procedures were conducted between 1400–1800 h. All procedures used in the study were approved by our institutional ethical committee.

Drug Discrimination Procedure

The procedure similar to the standard fixed-ratio 10 (FR10) drug discrimination paradigm (4), was essentially the same as the procedure used in our previous experiments with 5-HT_3 and NMDA receptor ligands (3,24). Four standard two-lever operant conditioning chambers (Coulbourn Instruments, Inc., Allentown, PA) consisted of modular test cages enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centred near the top of the front panel of the cage, which was also equipped with two response levers, separated by a liquid dipper. The liquid dipper presented sweetened milk in a 0.01 ml portion for 5 s during each operation. Experimental sessions and data recording made used of the Coulbourn L91-04 interface and L2T2 software package running on a IBM-PC compatible. Drug discrimination training began only after all the animals responded reliably on both levers under the FR1 conditions. The number of responses for each reinforcement was gradually increased from 1 to 10, and all subsequent training and testing sessions used the FR10 schedule of reinforcement. Rats were trained to press one lever following ethanol injections (1.0 g/kg, 10% v/v; IP) and to press the other lever following saline vehicle injections. Injections occurred 15 min prior to the start of 15-min sessions, which were conducted daily (Monday–Friday) under the alternating drug sequence (drug, drug, saline, saline, drug or saline, saline, drug, drug, saline). The animals continued to be trained under these conditions until they met, during 9 out of 10 consecutive sessions, the following two acquisition criteria: 1) 80% or more of the responses that occurred before the first reinforcement was made on the correct lever; and 2) during the remainder of the session, more than 90% of the responses were on the correct lever. After the animals had reached the criteria, dose–response and substitution tests were initiated. Test sessions were conducted once or twice times per week, with training sessions intervening during the remaining days. During test sessions the lever on which 10 responses accumulated first was defined as the selected lever. After lever selection the rat received subsequent reinforcements after pressing the selected lever only. To be tested, rats had to have reached the criteria for at least 5 days before the consecutive test. In addition, the data were excluded from the analysis if the rat failed to reach the criteria for at least 2 days after the test session. In dose–response tests, rats were tested after the administration of various doses of ethanol doses (0.25, 0.5, 0.75, and 1.0 g/kg; 10% v/v) 15 min before start of the test session. In substitution tests abecarnil (0.05–0.5 mg/kg), bretazenil (1.0–10.0 mg/kg), diazepam (0.5–2.5 mg/kg), and zolpidem (0.005–1.0 mg/kg), were administered IP 30 min before start of the test session.

Drugs

Ethanol (95%) was obtained from hospital pharmacy and diluted to the final concentration with physiological saline. Diazepam (Polfa, Warsaw, Poland) and bretazenil (Hoffmann–La Roche, Basel, Switzerland) were suspended in 1% Tween and administered in volumes of 2.0 ml/kg. Abecarnil (Schering AG, Berlin, Germany) and zolpidem (Synthelabo, Bagneux, France) were dissolved in saline and injected in volumes of 2.0 ml/kg.

Data Analysis

The percentage of ethanol-appropriate responding was calculated, using only the responses that occurred before the first reinforcement, by dividing the responses made on the ethanol-appropriate lever by the total number of responses on both levers, and multiplying the result by 100. The operational definition of partial stimulus substitution was 40% (or more) of responding on ethanol-appropriate lever. The operational definition of complete stimulus substitution was 80% (or more) of responding on the ethanol-appropriate lever (10). The response rates were calculated as the total number of responses (on both levers) during the session divided by the session time in seconds. Lever selection data (but not the re-

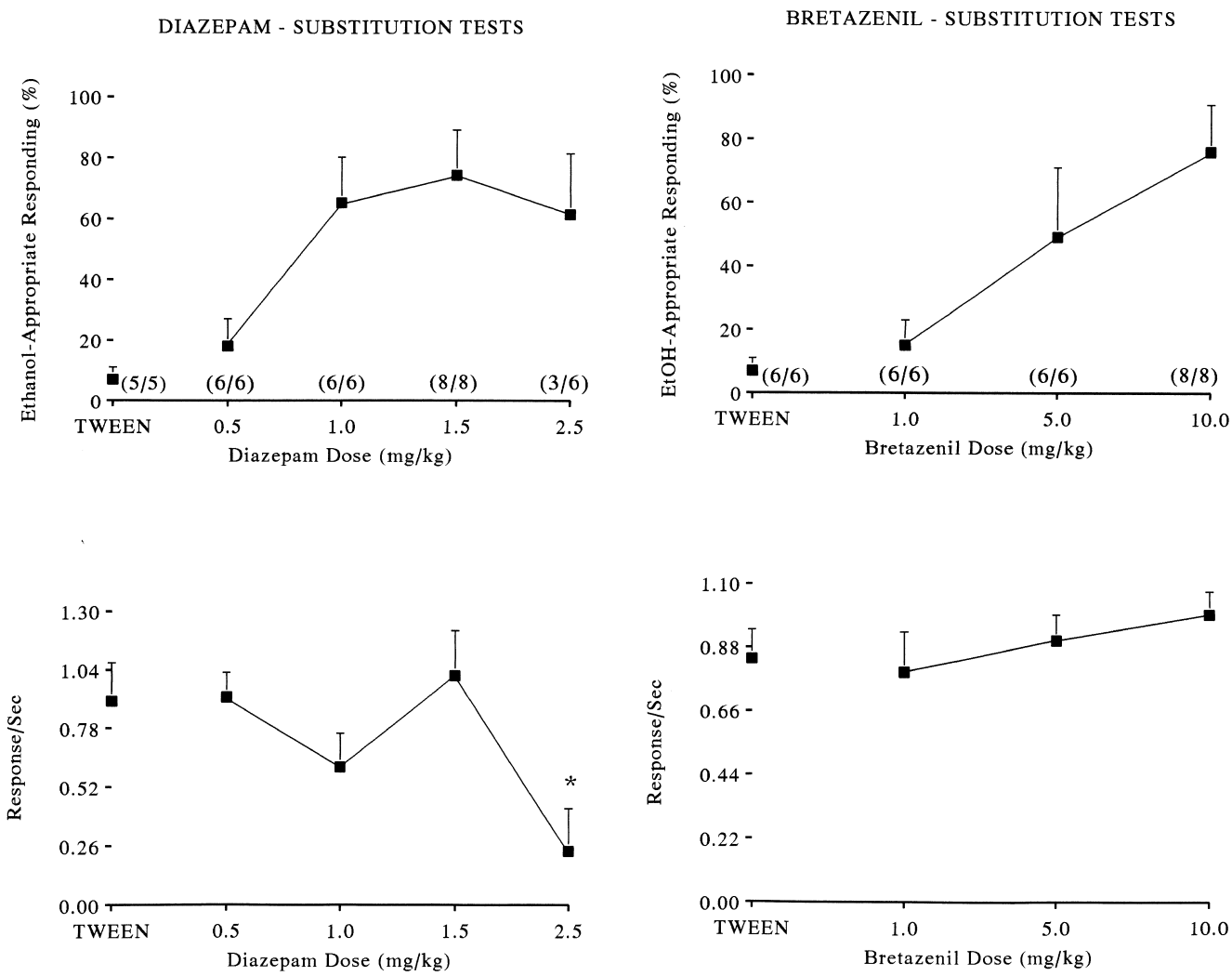

FIG. 1. Mean percentage (±SEM) of ethanol-appropriate responding (upper panel) and the mean (±SEM) response rate (lower panel) following ascending doses of diazepam and bretazenil in rats trained to discriminate between 1.0 g/kg ethanol and saline (*$p < 0.05$, *$p < 0.01$). *N/N*—number of rats completing the test session/number of rats tested.

sponse rate data) from the test session were not included if the rat failed to complete at least one FR10 on either lever in 15 min. ED50 and 95% confidence limits (C.L.) were calculated for the dose–response test according to the method of Litchfield and Wilcoxon (27). Students'*t*-test two tailed was used for comparing response rates from the test sessions.

RESULTS

All animals ($n = 12$) acquired the ethanol-saline discrimination (range: 27–54 training sessions). Once the discriminations were established, rats responded with stable accuracy on ethanol- or saline-appropriate lever (minimal mean value for 12 rats was: 92% for training sessions performed between the drug tests). ED_{50} calculated for the dose–response tests was: 0.51 g/kg (C.L.: 0.34–0.73). None of the ethanol doses influenced the response rate (data not shown).

Both diazepam and bretazenil dose dependently and almost completely (>75%) substituted for ethanol (Fig. 1, upper panel). Maximal substitution occurred at the doses that did not affect the response rate (Fig. 1, lower panel). Abecarnil failed to substitute for ethanol. Some ethanol-appropriate responding (although less than 40%) was observed in the range of doses (0.1–0.5 mg/kg) strongly reducing the rate of responding (Fig. 2). At best partial substitution was observed for zolpidem (<50%) but only at the dose (1.0 mg/kg) strongly suppressing the response rate (Fig. 2).

DISCUSSION

The results of the present study indicate that nonselective BZ receptor agonists, diazepam and bretazenil, generalize from ethanol in the rat. Diazepam and other nonselective full agonists at the BZ receptor, like chlordiazepoxide or midazolam, have been shown to substitute, either partially (1,2,14, 21,22) or fully (1,2,11,18,21), for ethanol in many previous studies using different animal species. Notably, our results reveal that also the partial agonist at the BZ receptor, bretaze-

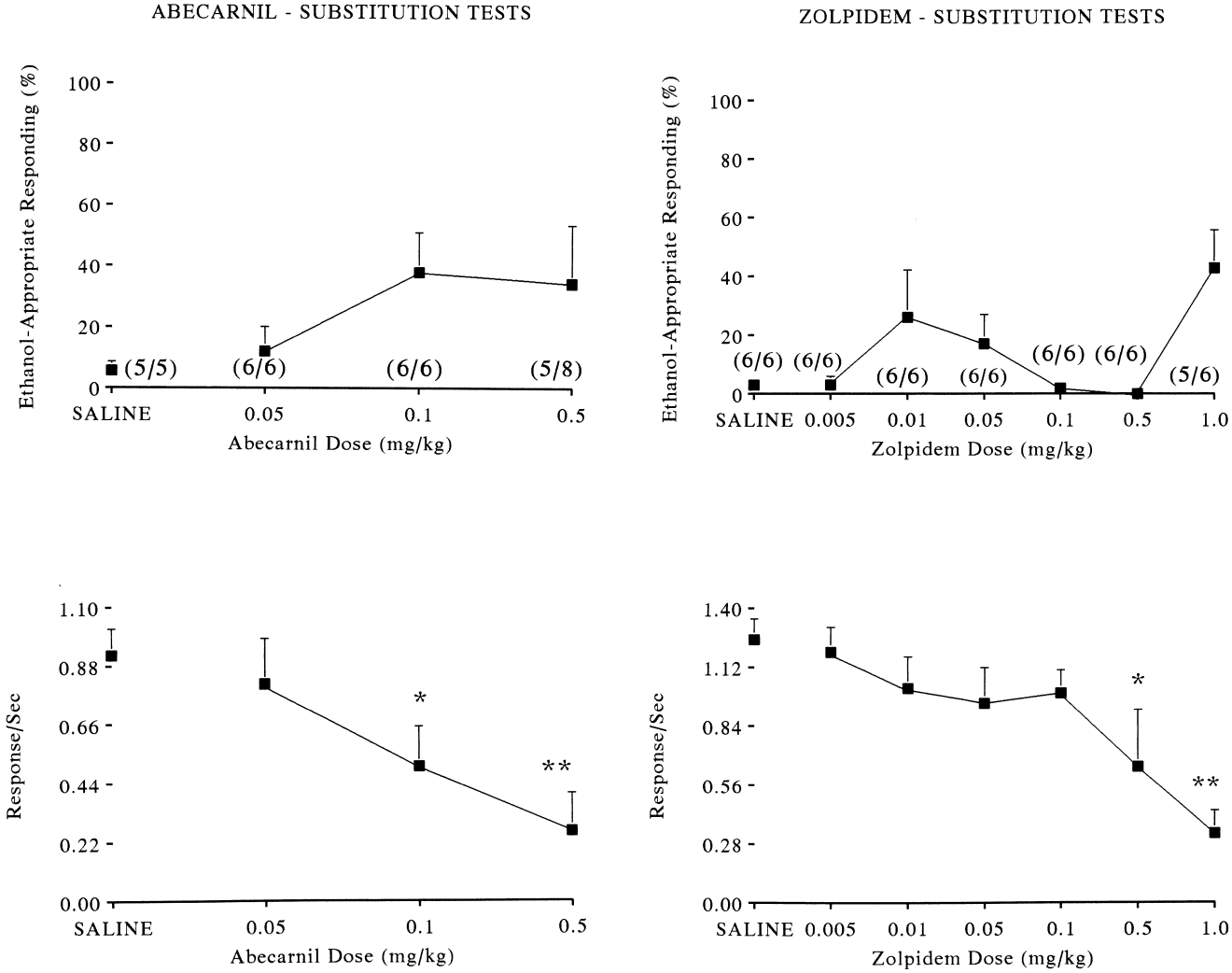

FIG. 2. Mean (±SEM) percentage of ethanol-appropriate responding (upper panel) and mean (±SEM) response rate (lower panel) following ascending doses of abecarnil and zolpidem in rats trained to discriminate 1.0 g/kg ethanol from saline (*$p < 0.05$, *$p < 0.01$). *N/N*—number of rats completing the test session/number of rats tested.

nil, may generalize from ethanol. As a matter of fact, bretazenil proved to be at least equally effective as diazepam when tested in rats trained to discriminate 1.0 g/kg ethanol from saline. However, our results do not exclude the possibility that bretazenil produces less ethanol-appropriate responding than diazepam in rats trained with a different dose of ethanol. Both diazepam and bretazenil were able to produce their maximal ethanol-like stimulus effects without any reduction of the response rate. Thus, these drugs may partially substitute for the ethanol cue at the doses that do not disrupt a normal operant behaviour. Several other drug discrimination studies have shown that ethanol-like stimulus effects produced by BZ receptor agonists are not accompanied by the rate suppressing effect [(1,14,22); but see also (18,21)].

In contrast, both abecarnil and zolpidem produced rather modest (<50%) ethanol-appropriate responding and only at the doses affecting significantly the rate of responding. These results support, although indirectly, the belief that the subjective profile of abecarnil and zolpidem differ from that of nonselective classical benzodiazepines (17,19,20,21,25,28). In agreement with our findings, abecarnil produced at best a partial substitution (40%) for the ethanol cue in rats trained to discriminate 1.0 g/kg ethanol from saline (18). Similarly, BZ1 receptor selective, partial agonist, alpidem, failed to generalize from ethanol in rats (18,21). Abecarnil is a partial agonist at the BZ receptors containing α_2 and α_5 subunits and a full agonist at the receptors with α_1 or α_3 subunits (12,16). Thus, abecarnil possesses full efficacy at the BZ1 receptor and at some of the BZ2 receptors (16). Importantly, some degree of selectivity towards the BZ1 receptor has also been reported for abecarnil (5,12). These results taken together with the findings from the present study (Fig. 2) suggest that stimulation of the $GABA_A$ receptor population with BZ1 receptor pharmacological characteristics is not sufficient to produce the ethanol-like discriminative stimulus effects in rats. This could lead to the hypothesis that $GABA_A$ receptors with BZ2 receptor pharmacological characteristics are mainly involved in the formation of the ethanol cue. Interestingly, it has been hypothesized on the basis of the corre-

lational studies, that the stimulus effects of chlordiazepoxide are mediated through BZ2 receptors (19,20). Alternatively, nonselective agonistic effects at different subtypes of the $GABA_A$ receptor complex are optimal for generalization from ethanol.

Concluding, the findings from this and previous studies (18,21) argue against the hypothesis, that the zolpidem-binding $GABA_A$receptor population (corresponding to the BZ1 receptor pharmacological properties) is preferentially involved in the central effects of ethanol (6,7). However, further studies using different in vivo procedures are needed to confirm or exclude the preferential involvement of any particular $GABA_A$ receptor population in central effects of ethanol.

ACKNOWLEDGEMENTS

A part of this article was presented on the Sixth Biennial Meeting of the European Behavioral Pharmacology Society, May 13–14, 1996, Cagliari, Italy. This work was supported by Institute of Psychiatry and Neurology, Warsaw, Poland (Grant No. 11/96). The authors thank Dr. S. Z. Langer (Synthelabo, France) for his gift of zolpidem.

REFERENCES

1. Ator, N. A.; Grant, K. A.; Purdy, R. H.; Paul, S. M.; Griffiths, R. R.: Drug discrimination analysis of endogenous neuroactive steroids in rats. Eur. J. Pharmacol. 241:237–243; 1993.
2. Azarov, A. W.; Szeliga, K. T.; Grant, K. A.: Assessment of $GABA_A$ ligands in male and female monkeys discriminating a low dose of ethanol. Alcohol. Clin. Exp. Res. 20:63A; 1996.
3. Bienkowski, P.; Stefanski, R.; Kostowski, W.: Competitive NMDA receptor antagonist, CGP 40116 substitutes for the discriminative stimulus effects of ethanol. Eur. J. Pharmacol. 314:277–280; 1996.
4. Colpaert, F.: Drug discrimination: Behavioural, pharmacological and molecular mechanisms of discriminative drug effects. In: Goldberg, S. R.; Stolerman, I. P., eds. Behavioural analysis of drug dependence. New York: Academic Press; 1986:161–193.
5. Costa, E.; Guidotti, A.: Benzodiazepines on trial: A research strategy for their rehabilitation. Trends Pharmacol. Sci. 17:192–200; 1996.
6. Criswell, H. E.; Simson, P. E.; Duncan, G. E.; McCown, T. J.; Herbert, J. S.; Morrow, A. L.; Breese, G. R.: Molecular basis for regionally specific action of ethanol on γ-aminobutyric acidA receptors: Generalization to other ligand-gated ion channels. J. Pharmacol. Exp. Ther. 267:522–537; 1993.
7. Criswell, H. E.; Simson, P. E.; Duncan, G. E.; Morrow, A. L.; Kier, W.; Breese, G. R.: Ethanol enhancement of iontophoretically applied GABA: Association with type-1 benzodiazepine receptor binding. Alcohol. Clin. Exp. Res. 16:376; 1992.
8. Grant, K. A.: Emerging neurochemical concepts in the actions of ethanol at ligand-gated ion channels. Behav. Pharmacol. 5:383–404; 1994.
9. Grant, K. A.; Azarov, A.; Bowen, C. A.; Mirkis, S.; Purdy, R. H.: Ethanol-like discriminative stimulus effects of the neurosteroid 3α-hydroxy-5α-pregnan-20-one in female *Macaca fascicularis* monkeys. Psychopharmacology (Berlin) 124:340–346; 1996.
10. Grant, K. A.; Colombo, G.: Discriminative stimulus effects of ethanol: Effect of training dose on the substitution of *N*-methyl-D-aspartate antagonists. J. Pharmacol. Exp. Ther. 264:1241–1247; 1993.
11. Hiltunen, A. J.; Järbe, T. U.: Discrimination of Ro 11-6896, chlordiazepoxide and ethanol in gerbils: Generalization and antagonism tests. Psychopharmacology (Berlin) 89:284–290; 1986.
12. Knoflach, F.; Drescher, U.; Scheurer, L.; Malherbe, P.; Möhler, H.: Full and partial agonism displayed by benzodiazepine receptor ligands at recombinant γ-aminobutyric $acid_A$ receptor subtypes. J. Pharmacol. Exp. Ther. 266:385–391; 1993.
13. Liljequist, S.; Engel, J.: Effects of GABAergic agonists and antagonists on various ethanol-induced behavioural changes. Psychopharmacology (Berlin) 78:71–75; 1982.
14. Lytle, D. A.; Egilmez, Y.; Rocha, B. A.; Emmett-Oglesby, M. W.: Discrimination of ethanol and of diazepam: Differential cross-tolerance. Behav. Pharmacol. 5:451–460; 1994.
15. Mereu, G.; Gessa, G. L.: Low doses of ethanol inhibit the firing of neurons in the substantia nigra pars reticulata: A GABAergic effect? Brain Res. 360:325–330; 1985.
16. Möhler, H.; Fritschy, J. M.; Löscher, B.; Rudolph, U.; Benson, J.; Benke, D.: The $GABA_A$ receptors. From subunits to diverse functions. In: Narahashi, T., ed. Ion channels, vol. 4. New York: Plenum Press; 1996:89–113.
17. Mumford, G. K.; Rush, C. R.; Griffiths, R. R.: Abecarnil and alprazolam in humans: Behavioral, subjective and reinforcing effects. J. Pharmacol. Exp. Ther. 272:570–580; 1995.
18. Sanger, D. J.: Substitution by NMDA antagonists and other drugs in rats trained to discriminate ethanol. Behav. Pharmacol. 4:523–528; 1993.
19. Sanger, D. J.; Benavides, J.: Discriminative stimulus effects of ω (BZ) receptor ligands: Correlation with in vivo inhibition of [^{3}H]-flumazenil binding in different regions of the rat central nervous system. Psychopharmacology (Berlin) 111:315–322; 1993.
20. Sanger, D. J.; Benavides, J.; Perrault, G.; Morel, E.; Cohen, C.; Joly, D.; Zivkovic, B.: Recent developments in the behavioral pharmacology of benzodiazepine (ω) receptors: Evidence for the functional significance of receptor subtypes. Neurosci. Biobehav. Rev. 18:355–372; 1994.
21. Sanger, D. J.; Zivkovic, B.: Discriminative stimulus effects of alpidem, a new imidazopyridine anxiolytic. Psychopharmacology (Berlin) 113:395–403; 1994.
22. Shelton, K. L.; Balster, R. L.: Ethanol drug discrimination in rats. Substitution with GABA agonists and NMDA antagonists. Behav. Pharmacol. 5:441–450; 1994.
23. Smith, B. R.; Segal, R. B.; Amit, Z.: Administration of a GABA antagonist selectively attenuates an ethanol-induced conditioned taste aversion. Pharmacol. Biochem. Behav. 33:269–271; 1989.
24. Stefanski, R.; Bienkowski, P.; Kostowski, W.: Studies on the role of 5-HT_3 receptors in the mediation of the ethanol interoceptive cue. Eur. J. Pharmacol. 309:141–147; 1996.
25. Stephens, D. N.; Schneider, H. H.; Kehr, W.; Andrews, J. S.; Rettig, K.-J.; Turski, L.; Schmiechen, R.; Turner, J. D.; Jensen, L. H.; Petersen, E. N.; Honore, T.; Bondo Hansen, J.: Abecarnil, a metabolically stable, anxioselective β-carboline acting at benzodiazepine receptors. J. Pharmacol. Exp. Ther. 253:334–343; 1990.
26. Suzdak, P. D.; Schwartz, R. D.; Skolnik, P.; Paul, S. M.: Ethanol stimulates gamma-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. Proc. Natl. Acad. Sci. USA 83:4071–4075; 1986.
27. Tallarida, R. J.; Murray, R. B.: Manual of pharmacological calculations. New York: Springer; 1987.
28. Witkin, J. M.; Acri, J. B.; Wong, G.; Gleeson, S.; Barrett, J. E.: Behavioral and biochemical characterization of benzodiazepine receptor partial agonists in pigeons. J. Pharmacol. Exp. Ther. 277: 87–96; 1996.