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ORIGINAL INVESTIGATION

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Positive allosteric modulators of the $GABA_A$ receptor: differential interaction of benzodiazepines and neuroactive steroids with ethanol

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Abstract Endogenous pregnane steroids, such as allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α,5α-P) and pregnanolone (3α-hydroxy-5β-pregnan-20-one; 3α,5β-P), allosterically modulate $GABA_A$ receptor function and exhibit behavioral effects similar to benzodiazepines, though acting at a distinct recognition site. Inasmuch as some positive allosteric modulators of $GABA_A$ receptor function exhibit profound interactions with ethanol, the effects of 3α,5α-P and 3α,5β-P were compared to those of two benzodiazepines, triazolam and diazepam, on the motor function of mice and rats when administered either alone or in combination with ethanol. All four test compounds exhibited dose-related impairment of motor function in the horizontal wire task in mice and the rotorod task in rats. Ethanol caused a marked enhancement of triazolam- and diazepam-induced motor impairment. In contrast, ethanol enhanced to a lesser extent the motor impairment induced by both neurosteroids in mice and not at all in rats. All four compounds increased ethanol-induced behavioral sleep time in mice, although the benzodiazepines did so at a much smaller fraction of their ataxic doses as compared to the neurosteroids. As one of the undesired side-effects of therapeutic use of benzodiazepines is their interaction with ethanol, development of neuroactive steroids as drugs may offer therapeutic advantages.

Key words Allopregnanolone · Pregnanolone · Neurosteroid · Neuroactive steroid · Motor behavior · Ethanol interaction · Benzodiazepine · Triazolam · Diazepam

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Introduction

Neuroactive steroids are positive modulators of the γ-aminobutyric $acid_A(GABA_A)$ Cl^- channel receptor complex (Paul and Purdy 1992; Gee et al. 1995) that selectively interact with a unique binding site on the $GABA_A$ receptor complex to potentiate inhibitory neurotransmission in the central nervous system (CNS). The neuroactive steroids pregnanolone (3α-hydroxy-5β-pregnan-20-one; 3α,5β-P) and allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α,5α-P) are endogenous metabolites of progesterone. In humans, progesterone is metabolically reduced to 3α,5β-P by 5β-reductase and to 3α,5α-P by 5α-reductase. Combined with the action of 3α-oxidoreductase, this metabolic pathway causes the loss of genomic activity and gain of CNS receptor mediated activity.

Many behavioral effects of neuroactive steroids are attributed to positive modulation of $GABA_A$ receptors. Specifically, pregnanolone and allopregnanolone exhibit anticonvulsant, anxiolytic and sedative behavioral effects (Belelli et al. 1989; Bitran et al. 1991; Wieland et al. 1991, 1995; Zimmerberg et al. 1994). These effects are similar to those exhibited by other positive allosteric modulators of the $GABA_A$ receptor complex such as benzodiazepines (Colpaert et al. 1976; Harvey 1985; Saano 1987). Further, benzodiazepines and neuroactive steroids have been demonstrated to share discriminative stimulus effects (Ator et al. 1993; Vanover 1997).

As an adverse side-effect, benzodiazepines have shown a great propensity for interaction with ethanol, producing marked decrements in psychomotor performance in both animals (Chan 1984; Hu et al. 1986; Barnhill et al. 1991) and humans (Sellers and Busto 1982; Chan 1984). Indeed, ethanol-induced motor impairment has been shown to be mediated via a GABAergic mechanism (Frye and Breese 1982; Liljequist and Engel 1982; Martz et al. 1983) and ethanol has been demonstrated to interact with the

$GABA_A$ receptor complex at the molecular level (Ticku et al. 1983; Ticku 1989; Buck and Harris 1990a,b). Thus, it is reasonable to speculate that other GABAergic positive modulators might enhance the effects of ethanol similar to the enhancement observed with benzodiazepines. Indeed, 3α,5β-P has been shown to enhance motor impairment, hypothermia, and hypnosis induced by ethanol in mice (Melchoir and Allen 1992). Further, evidence suggests that the interaction between ethanol and neuroactive steroids is mediated via the $GABA_A$ receptor (Majewska 1988). The purpose of the present study was to compare the magnitude of the interaction of neuroactive steroids and benzodiazepines with ethanol. The motor function of mice and rats treated with the neurosteroids, 3α,5α-P or 3α,5β-P, or the benzodiazepines, triazolam or diazepam, was evaluated alone and in the presence of ethanol. In addition, the extent of potentiation of ethanol-induced behavioral sleep by the benzodiazepines and neuroactive steroids was evaluated.

Materials and methods

Animals

Naive male rats (Sprague-Dawley) weighing 200–225 g and naive male mice (NSA) weighing 20–25 g were obtained from Harlan Sprague-Dawley, Inc. (San Diego, Calif., USA). Animals were housed in polycarbonate cages (two rats per cage; four mice per cage) containing sterilized bedding material (Sani-Chips; P.J. Murray, Montville, N.J., USA) and were kept in a room maintained at 23.0°C (±2.5°C) and on a 12 h:12 h light:dark cycle. Food (LM 485, Harlan Teklad, Orange, Calif., USA) and water were freely available. Animals were acclimated to housing conditions for a minimum of 4 days prior to experimentation. The "Principles of laboratory animal care" were followed in our AAALAC-accredited facilities for all experiments.

Apparatus and procedure

The rotorod test used a custom-built apparatus that consisted of an elevated drum (7.62 cm diameter) of textured surface that rotated at a constant speed (8 rpm). The height of the drum from the floor of the test apparatus was approximately 30 cm. Prior to administration of test substance, rats were trained to walk continuously on the drum for a period of 90 s. During testing, rats were given three opportunities to remain on the apparatus continuously for 1 min. Remaining on the apparatus was scored as a pass. Test drugs and ethanol were administered 30 min prior to testing.

The horizontal wire test also used a custom-built apparatus that consisted of a metal wire (2 mm diameter) suspended horizontally above a padded surface (25 cm). After the appropriate pretreatment interval following drug administration, mice were held by the base of the tail, their forepaws placed in contact with the wire, and then released. Mice were required to bring at least one hindpaw in contact with the wire within 10 s in order to be scored as a pass. Test drugs and ethanol were administered 30 min prior to testing.

Loss-of-righting reflex (LRR) was evaluated in mice following administration of a hypnotic dose of ethanol (3.0 g/kg, IP). Once animals were unable to right themselves twice within 30 s when placed on their backs on the bench-top, the time was recorded and mice were observed until the ability to right returned, whereupon the time was again noted. Righting was defined as two consecutive successful attempts within a 30-s period to stand on four paws when placed on their backs by the experimenter. Test drugs were administered concurrently with ethanol.

Data analysis

Results from rotorod and horizontal wire assays were treated quantally. To determine whether ethanol enhanced the motor impairment of benzodiazepines and neuroactive steroids, dose-response functions (n = 15–16/dose) of the benzodiazepines and neuroactive steroids were determined alone and in the presence of ethanol (1.0 g/kg for rats, 1.5 g/kg for mice). Each determination was based on two separate experiments (n = 7–8/dose) conducted on different days and the results summed. A dose which caused behavioral toxicity in half the animals (toxic dose; TD_{50}) was calculated based on the combined dose-response function by the method of Litchfield and Wilcoxon using PHARM/PCS version 4.2 software (Springer-Verlag, New York, USA). In addition, the 95% confidence intervals were calculated around each TD_{50}.

In the loss-of-righting assay, the number of animals exhibiting LRR, as well as the induction time and duration of LRR for each animal, were recorded. For the animals exhibiting LRR, the durations were averaged and the SEM were calculated and graphed. The minimum dose of a drug that, in combination with ethanol, exceeded twice the vehicle mean duration of ethanol-induced LRR was considered to be the minimum effective dose (MED) for enhancement of ethanol-induced behavioral sleep time.

Drugs

A vehicle of 50% hydroxypropyl-β-cyclodextrin (in saline) was used to dissolve 3α,5β-P, synthesized by AKZO-Diosynth (Oss, The Netherlands), 3α,5α-P, synthesized by CoCensys, Inc. (Irvine, Calif., USA), triazolam, generously supplied by Upjohn (Kalamazoo, Mich., USA), and diazepam, purchased from Sigma Chemical Company (St Louis, Mo., USA). Ethanol was purchased from Spectrum Chemical (Gardena, Calif., USA) and diluted with deionized water. All drugs were administered IP.

Results

The dose-response functions of the neuroactive steroids and benzodiazepines, alone and in combination with ethanol, in mice are shown in Fig. 1. Mice treated with 3α,5α-P and 3α,5β-P exhibited motor impairment in the horizontal wire assay with a TD_{50} of 24.7 mg/kg (95% CI: 16.7–36.7) and 20.3 mg/kg (14.3–28.9), respectively. The neurosteroids administered in combination with 1.5 g/kg ethanol showed greater motor incoordination with a TD_{50} of 5.8 mg/kg (3.9–8.5) for 3α,5α-P and 7.7 mg/kg (5.0–11.9) for 3α,5β-P, resulting in an ethanol potentiation ratio (TD_{50} alone/TD_{50} + ethanol) of 4 for 3α,5α-P and 3 for 3α,5β-P (Table 1). The benzodiazepines showed a similar, but even greater potentiation by ethanol (Table 1). Diazepam exhibited a TD_{50} of 1.2 mg/kg (0.9–1.5) administered alone and 0.10 mg/kg (0.07–0.14) administered in combination with ethanol, resulting in an ethanol potentiation ratio of 12. Further, triazolam

Table 1 Effects of neurosteroids and benzodiazepines in the horizontal wire assay in mice

Drug	TD_{50}^{a} alone	TD_{50}^{a} + 1.5 g/kg ETOH	Ratio TD_{50} alone/TD_{50} + ETOH)
3α,5α-P	24.7 (16.7–36.7)	5.8 (3.9–8.5)	4
3α,5β-P	20.3 (14.3–28.9)	7.7 (5.0–11.9)	3
Diazepam	1.2 (0.9–1.5)	0.10 (0.07–0.14)	12
Triazolam	0.21 (0.06–0.76)	0.01 (0.007–0.02)	21

[a]TD_{50} expressed as mg/kg (95%CI)

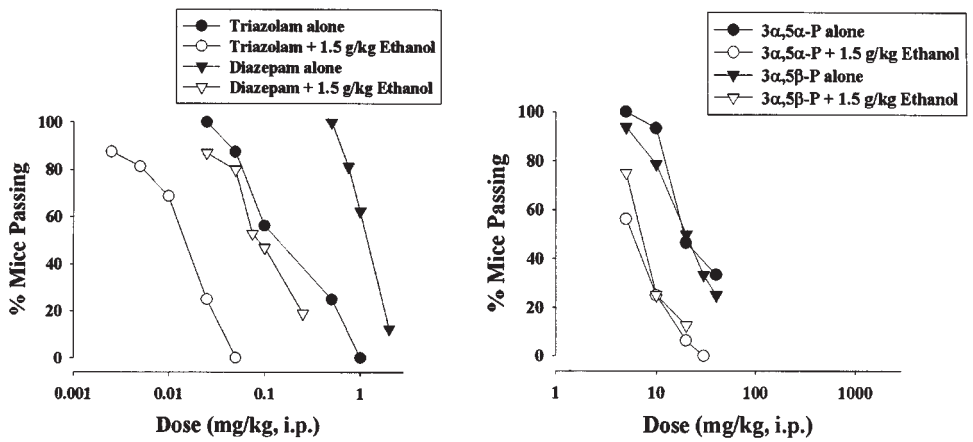

Fig. 1 Benzodiazepine- and neuroactive steroid-induced deficits alone and in combination with ethanol in the horizontal wire assay in mice. Percent mice passing is shown as a function of dose. Each point represents the data from 15–16 mice

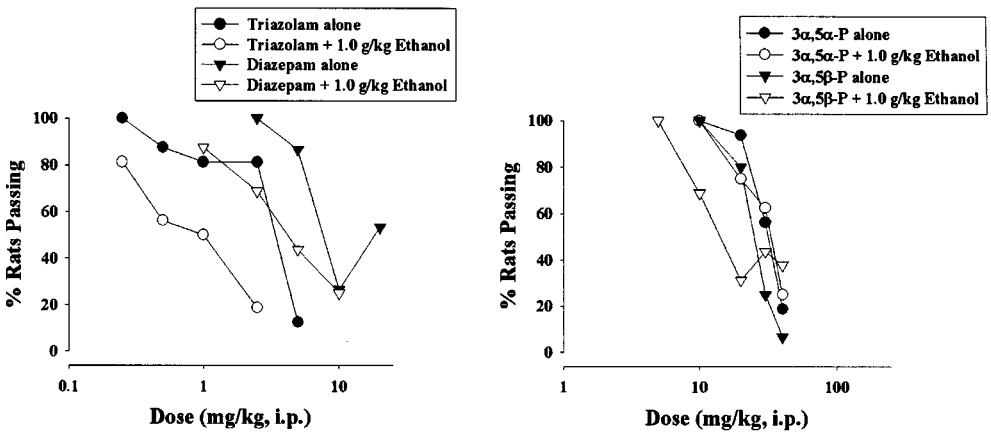

Fig. 2 Benzodiazepine- and neuroactive steroid-induced deficits alone and in combination with ethanol in the rotorod assay in rats. Percent rats passing is shown as a function of dose. Each point represents the data from 15–16 rats

exhibited a TD_{50} of 0.21 mg/kg (0.06–0.76) administered alone and 0.01 mg/kg (0.007–0.02) administered in combination with ethanol, resulting in an ethanol potentiation ratio of 21.

Rats tested in the rotorod assay showed motor impairment by the neurosteroids and benzodiazepines similar to that observed in mice (Fig. 2). Administered alone, triazolam was the most potent, with a TD_{50} of 3.3 mg/kg (1.9–5.5), followed by diazepam [TD_{50} = 12.5 mg/kg (8.3–18.7)], 3α,5β-P [TD_{50} = 23.3 mg/kg (17.2–31.6)], and 3α,5α-P [TD_{50} = 32.5 mg/kg (26.6–39.8)]. In combination with 1.0 g/kg ethanol, the TD_{50} of triazolam was 0.8 mg/kg (0.5–1.3) and the TD_{50} of diazepam was 4.4 mg/kg (2.7–7.1), resulting in ethanol potentiation ratios of 4 and 3, respectively (Table 2). In contrast, ethanol failed to potentiate the motor impairment induced by 3α,5α-P or 3α,5β-P in rats (Table 2). In combination with 1.0 g/kg ethanol, 3α,5α-P resulted in a TD_{50} of 33.0 mg/kg (25.7–42.7) and 3α,5β-P resulted in a TD_{50} of 22.8 mg/kg (16.1–32.4).

Ethanol (3.0 g/kg, IP, n = 9–10 mice/group) caused three to nine mice/group to lose the righting reflex (Fig. 3). For the mice exhibiting LRR, the mean duration of behavioral sleep ranged from 8.3 min (±2.9, SEM) to 24.0 min (±9.1) for the four vehicle control groups. The MED of triazolam (0.005–0.025 mg/kg, IP) to enhance ethanol-induced behavioral sleep was 0.01 mg/kg, IP. At this dose combination (0.01 mg/kg triazolam with 3.0 g/kg ethanol), six of nine mice slept for an average of 38.4 min (±21.6). Diazepam (0.1–0.5 mg/kg, IP) enhanced ethanol with its MED

Table 2 Effects of neurosteroids and benzodiazepines in the rotorod assay in rats

Drug	TD_{50}^{a} alone	TD_{50}^{a} + 1.0 g/kg ETOH	Ratio (TD_{50} alone/TD_{50} + ETOH)
3α,5α-P	32.5 (26.6–39.8)	33.0 (25.7–42.7)	1
3α,5β-P	23.3 (17.2–31.6)	22.8 (16.1–32.4)	1
Diazepam	12.5 (8.3–18.7)	4.4 (2.7–7.1)	3
Triazolam	3.3 (1.9–5.5)	0.8 (0.5–1.3)	4

[a]TD_{50} expressed as mg/kg (95% CI)

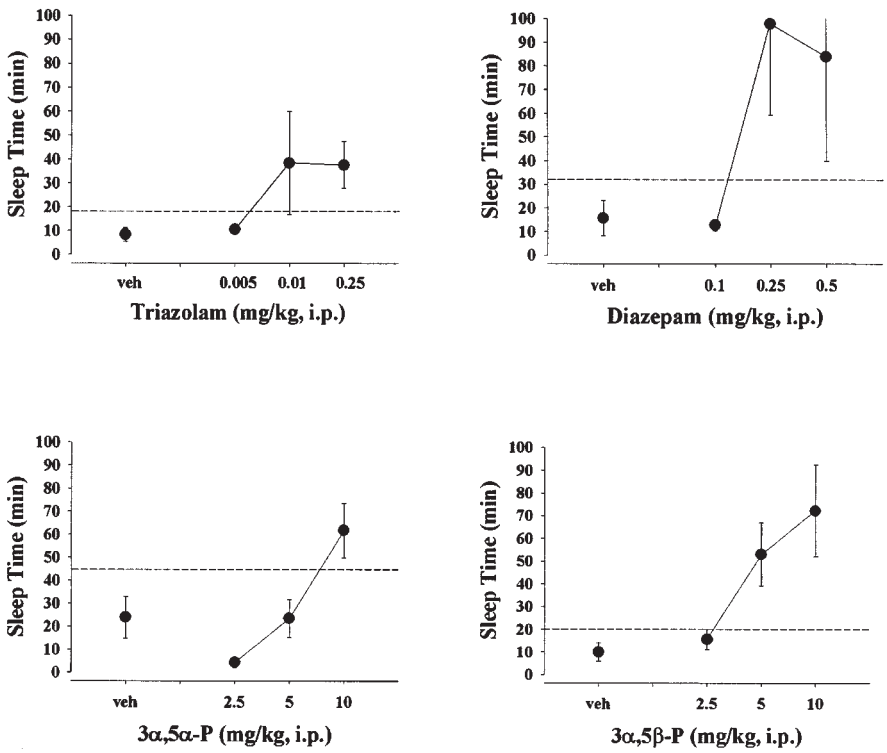

Fig. 3 Potentiation of ethanol-induced loss-of-righting by benzodiazepines and neuroactive steroids in mice. Sleep time (min) is shown as a function of dose of benzodiazepine or neuroactive steroid in combination with 3 g/kg ethanol. Each point represents the mean from a group of three to nine mice. Standard errors of the mean are shown by the *vertical lines*. The *horizontal dashed line* represents the criterion for enhancement of ethanol-induced sleep time (i.e., twice the vehicle mean duration)

of 0.25 mg/kg causing five of ten mice to exhibit LRR for an average sleep time of 97.9 min (± 38.65). The MEDs for 3α,5α-P (2.5–10.0 mg/kg, IP) and 3α,5β-P (2.5–10.0 mg/kg, IP) to enhance ethanol-induced behavioral sleep were 10.0 mg/kg and 5.0 mg/kg, respectively. The dose of 10.0 mg/kg 3α,5α-P together with 3.0 g/kg ethanol caused all nine mice injected to sleep an average of 61.8 min (±11.95), whereas 5.0 mg/kg 3α,5β-P with the ethanol caused nine of ten mice to sleep an average of 53.0 min (± 13.9).

Discussion

The present study confirms previous demonstrations of motor impairment induced by these neuroactive steroids and benzodiazepines in rodents (Facklam et al. 1992; Wieland et al. 1995). In both mice and rats, the benzodiazepines were more potent in motor impairment than the neuroactive steroids, triazolam being the most potent compound overall. The benzodiazepines appeared to be more potent in mice as compared to rats, whereas the neuroactive steroids exhibited similar potency in both species.

As measured by the horizontal wire assay in the mice, motor impairment induced by all four allosteric positive modulators studied was enhanced by co-administration of ethanol. These results are consistent with reports in the literature of ethanol enhancement of benzodiazepine- and neuroactive steroid-induced motor impairment in rodents (Hu et al. 1986; Melchoir and Allen 1992). However, ethanol had a greater effect on the benzodiazepines than on the neuroactive steroids in this assay. The effects of triazolam on horizontal wire performance were enhanced to the largest degree, with more than a 20-fold difference between its TD_{50}s in the presence and absence of ethanol. For diazepam alone, the TD_{50} was 12 times that in the presence of ethanol. In contrast, 3α,5α-P and 3α,5β-P showed less than a 5-fold enhancement of motor decrement produced by ethanol in mice.

The motor impairment induced by the benzodiazepines on rotorod performance in rats was also enhanced by ethanol. Of the drugs tested, triazolam

remained the most sensitive to ethanol interaction, with a 4-fold enhancement of motor impairment, followed by diazepam, with about a 3-fold enhancement. Ethanol had no effect on the rotorod deficits induced by 3α,5α-P and 3α,5β-P in rats. These results are consistent with previous reports of a lack of interaction with ethanol by the putative sedative-hypnotic neuroactive steroid CCD 3693 (Edgar et al. 1997) and a less than 2-fold enhancement of ethanol-induced motor impairment by the putative anxiolytic neuroactive steroid Co 6-0549 (Carter et al. 1995).

Overall, rats were less sensitive than mice regarding motor incoordination induced by $GABA_A$ positive modulators and enhancement by ethanol. Although it is possible that a difference in assay rather than species may underlie the differences observed between rats and mice in the present study, unpublished data suggest that TD_{50}s in mice measured by rotorod and horizontal wire assays are similar. Also, a previous study examining ethanol interactions with pentobarbital and phencyclidine reported a species difference between mice and rats (Wessinger and Balster 1987). The mechanism underlying such a species difference is unclear.

The duration of ethanol-induced behavioral sleep in mice was enhanced by each of the compounds tested. A comparison of the minimum dose of compound to enhance ethanol-induced sleep time and its ataxic dose provides an evaluation of the intensity of a compound's interaction with ethanol. 3α,5α-P exhibited a MED of 10.0 mg/kg with respect to enhancement of ethanol-induced sleep time, a dose approximately one-third its TD_{50} (24.7 mg/kg) in mice. Similarly, the MED of 3α,5β-P to enhance ethanol sleep time was 5.0 mg/kg, one-fourth its TD_{50} of 20.3 mg/kg. In contrast, the benzodiazepines enhanced ethanol-induced behavioral sleep time at much smaller fractions of their TD_{50}s. Diazepam enhanced ethanol with a MED of 0.25 mg/kg, a dose that was one-fifth its TD_{50} of 1.2 mg/kg. Triazolam exhibited the most robust interaction with ethanol, with a MED of 0.01 mg/kg, a dose that was 1/20th its ataxic TD_{50} of 0.21 mg/kg.

Neuroactive steroids bind to molecular sites on the $GABA_A$ receptor complex distinct from the benzodiazepine and barbiturate sites (Gee et al. 1988; Turner et al. 1989; Paul and Purdy 1992). The present results are consistent with the notion that, although modulation at the different sites causes some shared behavioral effects, the behavioral profiles resulting from differential modulation of the $GABA_A$ receptor complex are not identical. Indeed, previous investigations have demonstrated marked differences in the interaction of benzodiazepines and neuroactive steroids with the $GABA_A$ receptor following cessation of chronic ethanol consumption. Whereas ethanol withdrawal results in cross-tolerance to the anticonvulsant actions of diazepam, it produces sensitization to the anticonvulsant effects of 3α,5α-P (Devaud et al. 1996). In a similar manner, chronic ethanol administration has been shown to reduce thiopental-, but enhance 3α,5α-P-modulation of muscimol binding in rat brain (Negro et al. 1993). The present study suggests that ethanol also differentially modulates the interaction of benzodiazepines and neuroactive steroids with $GABA_A$ receptors following acute administration. Differential effects across $GABA_A$ receptor subunits by neuroactive steroids have been shown (Lan et al. 1991), and these effects may underlie the differential interaction of ethanol with neuroactive steroids compared to benzodiazepines.

Due to the endogenous nature of neurosteroids and the potential therapeutic usefulness of these and other, synthetic, neuroactive steroids (Gee et al. 1995), it is important thoroughly to understand the pharmacology of this class of compounds. Neuroactive steroids are currently being evaluated for therapeutic efficacy for the treatment of migraine, epilepsy, insomnia and anxiety disorders (Carter et al. 1995, 1997; Abrol et al. 1997; Edgar et al. 1997). The present study furthers the pharmacological characterization of neuroactive steroids by demonstrating a reduced propensity to interact with ethanol as compared with benzodiazepines. Clinically effective neuroactive steroids, therefore, may exhibit an improved side-effect profile over that of benzodiazepines.

References

Abrol S, Barrett JE, Carter R, Muth E, Rosenzweig-Lipson S (1997) Anxiolytic and discriminative stimulus effects of the neuroactive steroids Co 6-0549 and Co 2-6749 in pigeons. Soc Neurosci Abstr 23:960

Ator NA, Grant KA, Purdy PH, Paul SM, Griffiths RR (1993) Drug discrimination analysis of endogenous neuroactive steroids in rats. Eur J Pharmacol 241:237–243

Barnhill JG, Ciraulo DA, Greenblatt DJ, Faggart MA, Harmatz JS (1991) Benzodiazepine response and receptor binding after chronic ethanol ingestion in a mouse model. J Pharmacol Exp Ther 258:812–819

Belelli D, Bolger MB, Gee KW (1989) Anticonvulsant profile of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Eur J Pharmacol 166:325–329

Bitran D, Hilvers RJ, Kellogg CK (1991) Anxiolytic effects of 3α-hydroxy-5α-[β]-pregnan-20-one; endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res 561:157–161

Buck KJ, Harris RA (1990a) Benzodiazepine agonist and inverse agonist actions on $GABA_A$ receptor-operated chloride channels. I. Acute effects of ethanol. J Pharmacol Exp Ther 253:706–712

Buck KJ, Harris RA (1990b) Benzodiazepine agonist and inverse agonist actions on $GABA_A$receptor-operated chloride channels. II. Chronic effects of ethanol. J Pharmacol Exp Ther 253:713–719

Carter RB, Wieland S, Lan NC, Xia H, Belluzi JD, Stein L, Gee KW, Wood PL (1995) Anxiolytic properties of the novel orally-active neurosteroid Co 6-0549. Soc Neurosci Abstr 21:1345

Carter RB, Wood, PL, Weiland S, Hawkinson JE, Belelli D, Lambert JJ, White HS, Wolf HH, Mirsadeghi S, Tahir SH, Bolger MB, Lan NC, Gee KW (1997) Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3α-hydroxy-3β-methyl-5α-pregnan-20-one), a selective high-affinity steroid modulator of the $GABA_A$ receptor. J Pharmacol Exp Ther 280:1284–1295

Chan AWK (1984) Effects of combined alcohol and benzodiazepine: a review. Drug Alcohol Depend 6:341–349

Colpaert FC, Desmedt LKC, Janssen PAJ (1976) Discriminative stimulus properties of benzodiazepines, barbiturates and pharmacologically related drugs; relation to some intrinsic and anticonvulsant effects. Eur J Pharmacol 37:113–123

Devaud LL, Purdy RH, Finn DA, Morrow AL (1996) Sensitization of γ-aminobutyric acid$_A$ receptors to neuroactive steroids in rats during ethanol withdrawal. J Pharmacol Exp Ther 278:510–517

Edgar DM, Seidel WF, Gee KW, Lan NC, Field G, Xia H, Hawkinson JE, Wieland S, Carter RB, Wood PL (1997) CCD3693: an orally bioavailable analog of the endogenous neuroactive steroid, pregnanolone, demonstrates potent sedative hypnotic actions in the rat. J Pharmacol Exp Ther 282: 420–429

Facklam M, Schoch P, Bonetti EP, Jenck F, Martin JR, Moreau JL, Haefely WE (1992) Relationship between benzodiazepine receptor occupancy and functional effects in vivo of four ligands of differing intrinsic efficacies. J Pharmacol Exp Ther 261:1113–1121

Frye GD, Breese GR (1982) GABAergic modulation of ethanol-induced motor impairment. J Pharmacol Exp Ther 223:750–756

Gee KW, Bolger MB, Brinton RE, Coirini H, McEwen BS (1988) Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. J Pharmacol Exp Ther 246:803–812

Gee KW, McCauley LD, Lan NC (1995) A putative receptor for neurosteroids on the GABA$_A$ receptor complex: the pharmacological properties and therapeutic potential of epalons. Crit Rev Neurobiol 9:207–227

Harvey SC (1985) Hypnotics and sedatives. In: Gilman AG, Goodman LS, Rall TW, Murad F (eds) Goodman and Gilman's the pharmacological basis of therapeutics (seventh edition). Macmillan, New York, N.Y., pp 339–371

Hu WY, Reiffenstein RJ, Wong L (1986) Interaction between flurazepam and ethanol. Alcohol Drug Res 7:107–117

Lan NC, Gee KW, Bolger MB, Chen JS (1991) Differential responses of expressed recombinant human γ-aminobutyric acid$_A$receptors to neurosteroids. J Neurochem 57:1818–1821

Liljequist S, Engel J (1982) Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. Psychopharmacology 78:71–75

Majewska MD (1988) Interaction of ethanol with the GABA$_A$ receptor in the rat brain: possible involvement of endogenous steroids. Alcohol 5:269–273

Martz A, Deitrich RA, Harris RA (1983) Behavioral evidence for the involvement of γ-aminobutyric acid in the actions of ethanol. Eur J Pharmacol 89:53–62

Melchoir CL, Allen PM (1992) Interaction of pregnanolone and pregnenolone sulfate with ethanol and pentobarbital. Pharmacol Biochem Behav 42:605–611

Negro M, Casanova E, Chinchetru MA, Fernández-López A, Calvo P (1993) Differential effect of chronic ethanol treatment on barbiturate and steroid modulation of muscimol-binding to rat brain cortex. Neurosci Lett 158:83–86

Paul SM, Purdy RH (1992) Neuroactive steroids. FASEB J 6:2311–2322

Saano V (1987) GABA-benzodiazepine receptor complex and drug actions. Med Biol 65:167–173

Sellers EM, Busto U (1982) Benzodiazepines and ethanol: assessment of the effects and consequences of psychotropic drug interactions. J Clin Psychopharmacol 2:249–262

Ticku MK (1989) Ethanol and the benzodiazepine-GABA receptor ionophore complex. Experientia 45:413–418

Ticku MK, Burch TP, Davis WC (1983) The interactions of ethanol with the benzodiazepine-GABA receptor-ionophore complex. Pharmacol Biochem Behav 18 [Suppl 1]:15–18

Turner DM, Ransom RW, Yang S-J, Olsen RW (1989) Steroid anesthetics and naturally occurring analogs modulate the γ-aminobutyric acid receptor complex at a site distinct from barbiturates. J Pharmacol Exp Ther 248:960–966

Vanover KE (1997) Discriminative stimulus effects of the endogenous neuroactive steroid pregnanolone. Eur J Pharmacol 327:97–101

Wessinger WD, Balster RL (1987) Interactions between phencyclidine and central nervous system depressants evaluated in mice and rats. Pharmacol Biochem Behav 27:323–332

Wieland S, Lan NC, Mirasedeghi S, Gee KW (1991) Anxiolytic activity of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Brain Res 565:263–268

Wieland S, Belluzzi JD, Stein L, Lan NC (1995) Comparative behavioral characterization of the neuroactive steroids 3α-OH,5α-pregnan-20-one and 3α-OH,5β-pregnan-20-one in rodents. Psychopharmacology 118:65–71

Zimmerberg B, Brunelli SA, Hofer MA (1994) Reduction of rat pup ultrasonic vocalizations by the neuroactive steroid allopregnanolone. Pharmacol Biochem Behav 47:735–738