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Does the benzodiazepine antagonist Ro 15-1788 antagonize the action of ethanol?

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1 Ethanol aggravates benzodiazepine-induced central nervous depression by pharmacokinetic and/or pharmacodynamic interactions and Ro 15-1788 reverses promptly the hypnotic effects of benzodiazepines. We therefore studied the acute effects of Ro 15-1788 on the ethanol-induced sedation in six healthy male subjects.

2 Subsequently to an oral loading dose (0.54 g ethanol kg^{-1}) ethanol was infused for 4 h (0.15 g ethanol kg^{-1} h^{-1}) and steady state blood levels between 0.9 to 1.2 g l^{-1} were reached within 2 h. At steady state and during the elimination phase of ethanol an intravenous bolus of 0.5 mg Ro 15-1788 or placebo was administered in a randomized, double-blind crossover fashion.

3 The marked sedative effects of ethanol as assessed by visual analogue scales (2 to 6 fold increase in the sedation index), and choice reaction time (25 to 40% prolongation) were not affected by Ro 15-1788.

4 However, the pharmaco-EEG indicated that Ro 15-1788 seems to reverse transiently the ethanol-induced changes in total alpha, delta, and slow alpha bands.

5 There was no pharmacokinetic interaction between both agents since elimination of Ro 15-1788 ($t_{½}$ = 1.2 ± 0.7 h) and of ethanol (0.17 ± 0.02 g l^{-1} h^{-1}) were in good agreement with control values.

6 Thus, it could be concluded that Ro 15-1788 might affect for a short while the action of ethanol by interfering with the benzodiazepine receptors.

Keywords ethanol benzodiazepine antagonists pharmacodynamics pharmacokinetics drug interaction

Introduction

The depressant effects on the central nervous systems (CNS) as induced by benzodiazepines (BZD) and ethanol are generally very similar. It is well known that their common use produces enhanced cerebral depression. The exact mechanism of this clinically relevant drug interaction is poorly understood and it appears that pharmacokinetic as well as pharmacodynamic factors are involved (for review see Klotz, 1982). The pharmacologic effects of BZD are mediated by high-affinity stereospecific binding to the GABA-BZD-ionophore receptor complex and ethanol seems to enhance this BZD binding (Burch & Ticku, 1982).

The relatively specific benzodiazepine receptor antagonist Ro 15-1788 can reverse promptly and effectively BZD-induced sedative-hypnotic effects (Darragh *et al.*, 1981, 1982; Gaillard & Blois, 1983; Klotz *et al.*, 1985a). This acute action of Ro 15-1788 is of short duration, since the compound is rapidly eliminated with a half-life ($t_{½}$) of about 1 h (Klotz *et al.*, 1984). Whether Ro 15-1788 has also some pharmacodynamic effects of its own (e.g. sedative side-effects,

CNS-stimulant action), is a matter of discussion (Schöpf *et al.*, 1984; Ziegler *et al.*, 1985). It is conceivable that the action of Ro 15-1788 depends on the physiological state of the GABA-ergic system as proposed by Polc *et al.* (1982). In an activated GABA-system Ro 15-1788 might act as an inverse agonist with CNS-stimulant effects, during treatment with BZD as a specific antagonist and at subactive GABA-states as an agonist like the sedative BZD.

If the interaction between ethanol and BZD is mediated by the BZD receptor as indicated above, then Ro 15-1788 should affect also ethanol-induced CNS effects. Therefore we investigated under steady state conditions whether the BZD-antagonist Ro 15-1788 can reverse the pharmacological effects of ethanol in man.

Methods

Subjects and protocol

After approval by the ethics committee of our hospital, and written informed consent was obtained, six healthy, drug-free, non-smoking male volunteers (age range 25 to 45 years, weight 63 to 82 kg) participated in a placebo-controlled, double-blind, randomized crossover study.

Following a standard morning breakfast the subjects received an oral loading dose of 0.54 g ethanol kg^{-1} body weight dispensed in 200 ml orange juice. Subsequently a constant intravenous infusion of 0.15 g ethanol kg^{-1} h^{-1} was administered for 4 h. A bolus of placebo or Ro 15-1788 (0.5 mg) was injected twice: (a) 2.5 h after the start of the infusion (ethanol steady state condition) and (b) 2 h after stopping the infusion (ethanol elimination phase). Venous blood samples (8 ml) for the measurement of ethanol and Ro 15-1788 were drawn at zero time and 0.5, 1, 1.5, 2, 2.5, 2.75, 3, 3.25, 3.5, 4, 4.5, 5, 5.5, 6, 6.25, 6.5, 6.75, 7, 7.75 and 8 h after ethanol intake. At these time points also the response to ethanol was assessed.

Measurements

Blood levels of ethanol were measured in duplicate independently by a g.l.c. method (200 µl blood + internal standard propanol, 200 µl 0.3 M $Ba(OH)_2$, 200 µl 5% $ZnSO_4$ were vortexed, centrifuged and 4 µl supernatant injected on a porapak Q column (t = 160° C)) and by an automated fluorescencepolarization immunoassay (TDX-system, Abbott Diagnostic Division). Plasma concentrations of Ro 15-1788 were determined by a specific h.p.l.c.-assay with a lower limit of sensitivity of 2 ng ml^{-1} (Klotz *et al.*, 1984). The sedative-hypnotic effects of ethanol were assessed by three different methods:

(a) a subjective sedation index was formed from five visual analogue scales (length each 10 cm, maximal sedative score 50 cm)
(b) choice reaction time (RT1 and RT2) was measured by the Leeds psychomotor tester (mean of 10 trials)
In the choice reaction time apparatus the subject is required to scan an array of six small lights which are illuminated on a random basis. As soon as the subject detects the light (RT1) he is expected to touch the appropriate response button to extinguish the light (RT2). The latency of this response is an assessment of the integrity of the sensorimotor system and an accurate measure of psychomotor performance.
(c) EEG (central derivatives *vs* mastoid) was monitored for 3 min by an EEG amplifier (time constant 0.3 s, low-pass 30 Hz). The pulse code modulated tape-stored EEG recordings were evaluated by analogue to digital conversion (250 points s^{-1}), data tapering and transformation to frequency domain by Fourier algorithm. Subsequently log power values were calculated and integrated over six epochs of 15 s; eight different EEG wave band ranges were selected (see Table 3). Artifacts were controlled by visual inspection.

Elimination rate of ethanol and Ro 15-1788 was individually calculated from the corresponding terminal slopes by linear regression analysis. Relationships between blood ethanol concentrations and the three pharmacodynamic measurements (see a–c) were evaluated by application of the individual and mean data to the sigmoidal E_{max}-model and by linear regression analysis (Holford & Sheiner, 1981). Results are expressed as mean ± s.d. and comparisons between placebo and Ro 15-1788 were made using the paired Student's *t*-test and ANOVA.

Results

Pharmacokinetics

The applied loading/infusion dosage regimen resulted in steady state blood levels of ethanol in the range of 0.9 to 1.2 g l^{-1} which were achieved after about 2 h (see Figure 1). The coadministration of Ro 15-1788 did not change the concentration-time profile of ethanol either at steady state or during its elimination phase (see Figure 1). The slope of elimination varied across individuals between 0.15 and 0.18 g l^{-1} h^{-1} with mean values of 0.172 ± 0.017 g l^{-1} h^{-1} (+placebo) and

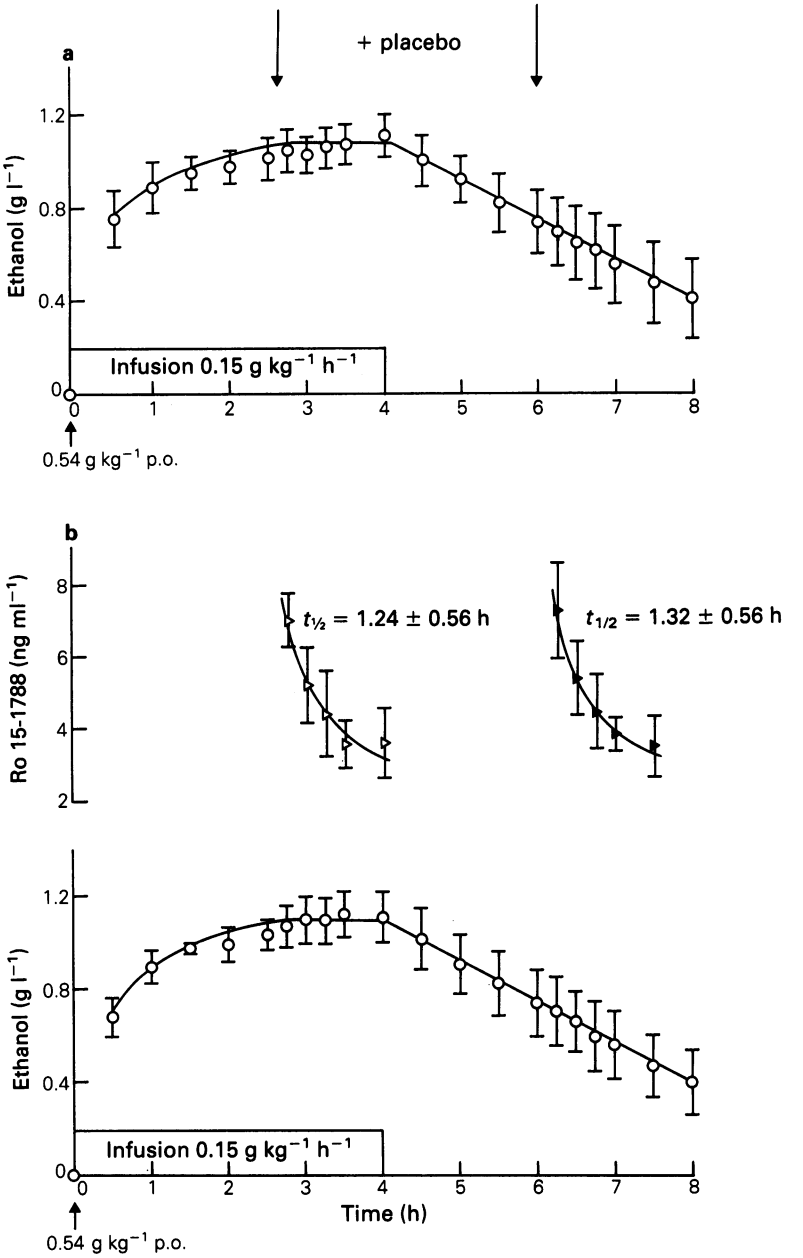

Figure 1 Ethanol blood concentration-time profiles (mean ± s.d.) in six healthy male volunteers following an oral loading of 0.54 g kg^{-1} with constant intravenous infusion (0.15 g kg^{-1} h^{-1}) for 4 h. (a) Placebo was injected twice 2.5 and 6 h after start of infusion; (b) Ro 15-1788 (0.5 mg i.v.) was coadministered twice at 2.5 and 6 h (see arrows) and its plasma levels monitored concomitantly.

0.168 ± 0.018 g l^{-1} h^{-1} (+Ro 15-1788). Likewise, the elimination half-life ($t_{½}$) of Ro 15-1788 was not altered by ethanol (Figure 1). Thus, a pharmacokinetic interaction between both agents can be excluded.

Pharmacodynamics

Almost in parallel to the rising blood levels of ethanol RT1 and RT2 were significantly prolonged, subjects felt significantly more sedated

and a clear slowing in the EEG could be observed. During the 12 trials in the six subjects tested the maximal sedative-hypnotic effects of ethanol preceded the peak blood levels only in one individual. In general, the CNS-depressant effects of ethanol were most pronounced when maximal, i.e. steady state blood levels were reached. As can be seen from the individual values of Table 1, sedation index increased several fold and choice reaction time was prolonged by up to 74%. The most significant individual association between ethanol blood concentrations and its sedative-hypnotic action could be seen on visual analogue scales which formed the sedation index (linear correlation coefficients varied between 0.4 and 0.8). In addition to this simple test, a significant relationship to the measurements of RT1 and RT2 could be derived from the mean values (Table 2), thereby all data could be fitted best to a sigmoidal E_{max}-model (see Figure 2). Half-maximal effective concentrations (EC_{50}) were in the range of 0.8 to 1.1 g l^{-1}.

The EEG indicated also ethanol-induced alterations (see Table 3a), e.g. an increase in theta-and slow-alpha-bands according to a marked decrease in fast alpha-bands. In the two psychometric tests applied, irrespective of the time of injection, no significant differences between the co-administration of placebo and Ro 15-1788 could be substantiated (see also Figure 2). However, in the EEG some effects are remarkable: Directly after the injection Ro 15-1788 appeared to reverse transiently the ethanol-induced changes (see Table 3b, line 1, 2, 4 and 5) whereas placebo had no effect at this time. During the elimination phase of ethanol Ro 15-1788 seemed to express a slight and short-lasting central activating effect directly after the injection (see Table 4b, line 7 and 8). Thus, in the doses applied, an interaction between ethanol

Table 1 CNS-depressant effects of ethanol (EtOH) at the time of its maximal blood concentration

	Sedation index (cm)		*RT1 (ms)*		*RT2 (ms)*	
Subject	*Control*	*at peak EtOH*	*Control*	*at peak EtOH*	*Control*	*at peak EtOH*
A.R.	+Ro 13.4	34.3	341	441	498	638
	+Pl 16.4	37.7	334	413	507	571
D.G.	+Ro 1.2	19.5	338	340	398	428
	+Pl 0.6	12.8	331	352	395	686
B.R.	+Ro 8.6	18.2	261	332	438	476
	+Pl 11.0	23.6	326	462	418	636
H.K.	+Ro 10.8	17.8	305	404	444	630
	+Pl 14.9	21.7	310	497	500	705
U.K.	+Ro 2.6	35.2	295	352	397	557
	+Pl 4.1	25.6	273	344	386	511
F.S.	+Ro 7.1	20.9	345	439	426	626
	+Pl 10.0	21.3	386	402	485	524
Mean	+Ro 7.3 ± 4.7	24.3 ± 8.2	314 ± 33	384 ± 50	434 ± 37	559 ± 89
± s.d.	+Pl 9.5 ± 6.1	23.8 ± 8.1	327 ± 37	412 ± 60	449 ± 55	601 ± 83

ANOVA: (effects of EtOH) sedation index: $P < 0.01$; RT1: $P < 0.01$; RT2: $P < 0.01$
Differences between controls: NS
Differences (Ro *vs* Pl) of EtOH's peak effects: NS
Ro: The benzodiazepine antagonist Ro 15-1788 (0.5 mg) or placebo (Pl) was given—see methods

Table 2 Effect-kinetic estimates of ethanol from the mean data of six healthy male subjects as analyzed by the sigmoidal E_{max}-model and linear regression analysis

	Ethanol + placebo			*Ethanol + Ro 15-1788*		
	Sedation	*RT1*	*RT2*	*Sedation*	*RT1*	*RT2*
E_{max}-model:						
E_{max}	17.2	123	134	16.8	114	137
EC_{50} (g l^{-1})	0.8	1.2	1.1	0.4	0.9	0.8
r value	0.92	0.78	0.75	0.87	0.83	0.75
(*P* value)	(< 0.001)	(< 0.001)	(< 0.001)	(< 0.001)	(< 0.001)	(< 0.001)
Linear correlation coefficient	0.77	0.58	0.64	0.77	0.56	0.54

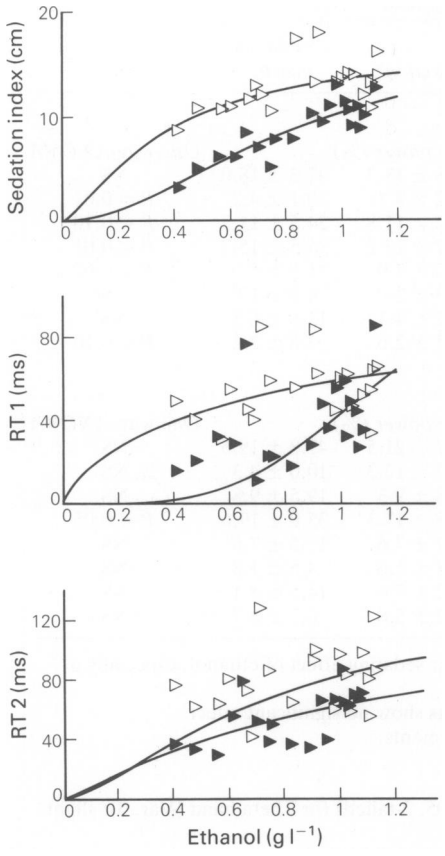

Figure 2 Relationships between the mean data of blood ethanol concentrations and its pharmacodynamic response in six healthy subjects as assessed at the different blood sampling times by the clinical application of the pharmacokinetic-pharmacodynamic sigmoidal E_{max}-model. Response on the ordinate is represented as unit changes from the predrug score (delta method). ▷ Ro 15-1788, ▶ placebo.

and the benzodiazepine antagonist seems possible.

Discussion

From our interaction study it is obvious that the CNS-depressant effects of ethanol are related to their blood levels and that there exists a close relationship between both time profiles (see Figure 2). In the literature a difference in the time pattern between blood ethanol concentrations and pharmacological response is discussed (Radlow & Hurst, 1985), which might be due to phenomena, such as sensitization, acute tolerance or presence of a drug equilibrium delay. However, these temporal modifications should result in hysteresis curves when evaluating our data by pharmacokinetic-pharmacodynamic modelling. Since under our steady state conditions no hysteresis effect could be observed by the three different tests applied, it might be concluded that the effects of ethanol are primarily concentration-dependent.

This clinical investigation demonstrates that no pharmacokinetic interaction between ethanol and Ro 15-1788 exists. Thereby the rate of ethanol elimination (0.17 g l^{-1} h^{-1}) in our healthy male subjects is very similar to other classical calculations of ethanol disposition (0.2 g l^{-1} h^{-1}; Rangno *et al.*, 1981). Similarly, $t_{½}$ of Ro 15-1788 (1.3 h) is within the range of previous studies (Klotz *et al.*, 1984, 1985b).

Whereas the BZD antagonist Ro 15-1788 competes with BZD for their binding sites at the GABA-BZD-ionophore receptor complex leading to a reversal of BZD-induced sedation or sleep (Darragh *et al.*, 1981, 1982; Gaillard & Blois, 1983; Klotz *et al.*, 1985a), this compound could not amplify ethanol-induced CNS effects as assessed by subjective visual analogue scales and choice reaction time. Thus, it could be concluded that in man under clinically relevant steady state conditions ethanol binding sites in the CNS must be different from those for BZD.

However, the EEG indicated a transient reversal of the ethanol's action after the injection of a small bolus of Ro 15-1788 (see Table 3b). The short-lasting EEG-changes induced by the second injection of Ro 15-1788 (see Table 4b) at somewhat lower ethanol blood levels (see Figure 1) would suggest a weak central activating potency. It could be concluded that the BZD-antagonist Ro 15-1788 possesses under such experimental conditions also some inverse agonistic potency. It is conceivable that the EEG might be a more sensitive indicator than both psychometric tests applied for CNS-activity and drug effects, respectively. The observed short duration of the antagonistic effect can be explained by the low dose and the fast elimination of Ro 15-1788. A higher dose might have had more pronounced effects which subsequently could have been also verified by the other tests. Thus, it could be concluded that the very short-lasting action of Ro 15-1788 is dependent on the time of its application and the concentrations of ethanol as well as on the pharmacodynamic model/assessment.

Our psychometric results are in good agreement with experimental data, e.g. behavioural studies in squirrel monkeys indicated that the effects of ethanol were not antagonized by Ro 15-1788 (Barrett *et al.*, 1985), Similarly, in rats

Table 3 EEG-data Ro 15-1788-ethanol interaction

Mean ± s.d.	*Baseline*	*2 h ethanol†*	*Injection**	*25 min after injection*	
a Injection of placebo and effect of ethanol on EEG-data					
Frequency bands:			*Relative power (%)*		*One-way-ANOVA*
1. 7.5 to 12.5 Hz:	43.2 ± 20.5	46.6 ± 19.9	43.8 ± 18.7	47.3 ± 18.0	NS
2. 1.5 to 3.5 Hz:	17.3 ± 8.1	11.0 ± 4.8	12.2 ± 7.1	10.1 ± 4.2	$P = 0.01$
3. 3.5 to 7.5 Hz:	18.0 ± 8.7	23.3 ± 10.8	24.2 ± 11.8	24.5 ± 11.7	$P < 0.10$
4. 7.5 to 10.0 Hz:	21.3 ± 12.6	34.7 ± 16.9	31.2 ± 13.2	35.5 ± 15.7	$P < 0.01$
5. 10.0 to 12.5 Hz:	21.3 ± 15.1	12.0 ± 8.0	12.2 ± 7.9	11.5 ± 8.0	$P = 0.01$
6. 12.5 to 14.5 Hz:	4.2 ± 2.5	4.3 ± 1.8	4.3 ± 2.0	4.2 ± 1.9	NS
7. 12.5 to 20.0 Hz:	13.3 ± 4.4	12.8 ± 6.9	13.2 ± 4.1	12.8 ± 6.3	NS
8. 20.0 to 32.0 Hz:	6.8 ± 2.9	4.8 ± 3.8	5.2 ± 2.6	3.8 ± 2.2	$P < 0.10$
b Injection of Ro 15-1788					
Frequency bands:			*Relative power (%)*		*One-way-ANOVA*
1. 7.5 to 12.5 Hz:	41.8 ± 19.1	46.0 ± 19.0	40.7 ± 21.5	47.8 ± 19.6	NS
2. 1.5 to 3.5 Hz:	15.3 ± 6.0	10.8 ± 3.3	15.2 ± 12.3	10.0 ± 4.3	NS
3. 3.5 to 7.5 Hz:	16.2 ± 6.7	19.2 ± 7.5	19.8 ± 9.3	19.5 ± 9.8	NS
4. 7.5 to 10.0 Hz:	21.0 ± 9.3	33.5 ± 17.9	25.8 ± 17.3	35.0 ± 19.6	$P < 0.05$
5. 10.0 to 12.5 Hz:	20.7 ± 15.2	12.5 ± 7.7	14.7 ± 7.6	12.5 ± 7.6	NS
6. 12.5 to 14.5 Hz:	4.5 ± 2.3	4.3 ± 1.9	5.3 ± 1.6	4.5 ± 1.8	NS
7. 12.5 to 20.0 Hz:	14.2 ± 5.3	14.2 ± 7.8	15.2 ± 7.6	14.5 ± 8.1	NS
8. 20.0 to 32.0 Hz:	10.7 ± 8.1	8.2 ± 6.6	7.2 ± 5.8	6.7 ± 6.7	NS

One-way-ANOVA (repeated measurement) indicate a clear sedating effect of ethanol, especially a shifting to slower frequency bands
Two-way-ANOVA (interaction) including all measurements show no significant effect
Statistical tests between a and b at single points of measurements:
at baseline: no significant differences
at 2 h ethanol†: no significant differences
at time soon after injection*: significant difference ($P < 0.05$, 2-tailed) for Theta band (Var. 3); slight difference ($P < 0.10$, 2-tailed) for spindle activity (Var. 6)
at 25 min after injection: no significant differences
By regarding the raw values there seems to be a slight antagonistic effect of Ro 15-1788 soon after injection of the drug (Table 3b) although statistically insignificant: In Var. 1 and 2 the values after injection (row 3) are very similar to those at baseline, in Var. 4 and 5 there seems to be a trend in direction to the baseline values.

the increased punishment response produced by ethanol was not altered by Ro 15-1788 (Liljequist & Engel, 1984).

β-Carbolines bind to the BZD-receptor complex as so-called inverse agonists because they have potent anxiogenic properties (Polc *et al.*, 1982; Dorow *et al.*, 1983). It was suggested that acetaldehyde, the primary metabolite of ethanol, can condense with serotonin to form a β-carboline (Kemperman, 1983). Thus, theoretically such β-carbolines could have been formed during our ethanol infusion and their anxiogenic action mediated by the BZD-receptor could have been prevented by Ro 15-1788.

Recently in anaesthetised rats cortical cerebral blood flow and cerebral oxygen consumption were determined. Ethanol potentiated the cerebral effects of midazolam. Since β-carboline reversed not only midazolam effects but also the interaction of midazolam plus ethanol, it was concluded that the potentiating effect of ethanol was produced by an effect at the BZD receptor (van Gorder *et al.*, 1985). This assumption is supported by *in vitro* binding studies indicating that ethanol enhances BZD-binding (Burch & Ticku, 1982). However, it should be mentioned that in both studies very high ethanol concentrations (range 46 to 500 mg 100 ml^{-1}) were involved.

Table 4 EEG-data Ro 15-1788-ethanol interaction during elimination phase of ethanol

Mean ± s.d.	*1.5 h post ethanol*	*Injection*	*15 min after injection*	
a Injection of placebo				
Frequency bands:		*Relative power (%)*		*One-way-ANOVA*
1. 7.5 to 12.5 Hz:	45.3 ± 14.9	40.2 ± 12.9	45.7 ± 17.1	NS
2. 1.5 to 3.5 Hz:	11.8 ± 3.3	14.2 ± 4.8	12.0 ± 4.3	NS
3. 3.5 to 7.5 Hz:	23.5 ± 8.9	26.0 ± 10.4	25.2 ± 11.8	NS
4. 7.5 to 10.0 Hz:	32.8 ± 13.2	28.2 ± 14.8	32.3 ± 13.4	NS
5. 10.0 to 12.5 Hz:	12.7 ± 8.3	12.2 ± 5.0	13.2 ± 8.0	NS
6. 12.5 to 14.5 Hz:	4.5 ± 2.1	5.0 ± 1.9	4.3 ± 1.8	NS
7. 12.5 to 20.0 Hz:	14.0 ± 6.2	14.0 ± 5.4	13.3 ± 6.9	NS
8. 20.0 to 32.0 Hz:	4.8 ± 2.1	5.8 ± 2.8	3.8 ± 1.5	$P < 0.10$
b Injection of Ro 15-1788				
Frequency bands:		*Relative power (%)*		*One-way-ANOVA*
1. 7.5 to 12.5 Hz:	46.3 ± 14.6	42.3 ± 11.6	49.3 ± 16.9	NS
2. 1.5 to 3.5 Hz:	13.8 ± 5.8	14.2 ± 4.4	11.0 ± 4.7	NS
3. 3.5 to 7.5 Hz:	21.3 ± 6.4	20.2 ± 4.4	18.7 ± 6.2	NS
4. 7.5 to 10.0 Hz:	31.2 ± 13.6	27.2 ± 12.1	34.0 ± 16.0	$P = 0.06$
5. 10.0 to 12.5 Hz:	15.0 ± 9.8	15.2 ± 5.8	15.3 ± 8.3	NS
6. 12.5 to 14.5 Hz:	5.0 ± 2.0	5.5 ± 2.5	5.2 ± 2.1	NS
7. 12.5 to 20.0 Hz:	13.8 ± 7.7	16.3 ± 6.0	15.5 ± 6.9	$P < 0.05$
8. 20.0 to 32.0 Hz:	4.7 ± 1.8	7.2 ± 2.6	5.5 ± 2.7	$P < 0.05$

Two-way-ANOVA (Interaction) show no significant effect
One-way-ANOVA table a has no significant effect
One-way-ANOVA table b indicate a slight central activating effect of Ro 15-1788 with an increase in Beta-1-band and Beta-2-Band and a transient decrease in Alpha-1-band.
No significant differences between a and b at single points

In conclusion, the specific benzodiazepine antagonist Ro 15-1788 appears to have some very short-lasting potency to reverse ethanol-induced CNS-depressant effects. Thus in the well-known and clinically relevant interaction between ethanol and BZD a (in)-direct event at the BZD-GABA-ionophore receptor complex cannot be excluded.

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