

The role of α_1 and α_5 subunit-containing GABA_A receptors in motor impairment induced by benzodiazepines in rats

Marija Milić^a, Jovana Divljaković^a, Sundari Rallapalli^b, Michael L. van Linn^b, Tamara Timić^a, James M. Cook^b and Miroslav M. Savić^a

Benzodiazepines negatively affect motor coordination and balance and produce myorelaxation. The aim of the present study was to examine the extent to which populations of γ -aminobutyric acid A (GABA_A) receptors containing α_1 and α_5 subunits contribute to these motor-impairing effects in rats. We used the nonselective agonist diazepam and the α_1 -selective agonist zolpidem, as well as nonselective, α_1 -subunit and α_5 -subunit-selective antagonists flumazenil, β CCt, and XLI093, respectively. Ataxia and muscle relaxation were assessed by rotarod and grip strength tests performed 20 min after intraperitoneal treatment. Diazepam (2 mg/kg) induced significant ataxia and muscle relaxation, which were completely prevented by pretreatment with flumazenil (10 mg/kg) and β CCt (20 mg/kg). XLI093 antagonized the myorelaxant, but not the ataxic actions of diazepam. All three doses of zolpidem (1, 2, and 5 mg/kg) produced ataxia, but only the highest dose (5 mg/kg) significantly decreased the grip strength. These effects of zolpidem were reversed by β CCt at

doses of 5 and 10 mg/kg, respectively. The present study demonstrates that α_1 GABA_A receptors mediate ataxia and indirectly contribute to myorelaxation in rats, whereas α_5 GABA_A receptors contribute significantly, although not dominantly, to muscle relaxation but not ataxia. *Behavioural Pharmacology* 23:191–197 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Behavioural Pharmacology 2012, 23:191–197

Keywords: ataxia, grip strength, muscle relaxation, rat, rotarod

^aDepartment of Pharmacology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia and ^bDepartment of Chemistry and Biochemistry, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin, USA

Correspondence to Dr Miroslav M. Savić, PhD, Department of Pharmacology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia
E-mail: miroslav@pharmacy.bg.ac.rs

Received 26 October 2011 Accepted as revised 9 December 2011

Introduction

Benzodiazepines (BZs) were introduced into clinical practice in the beginning of the 1960s and, since then, have been widely prescribed as anxiolytic, hypnotic, anti-convulsant, and myorelaxant drugs. During the 1990s, it became clear that the pharmacological effects of BZs are mediated by positive modulation of four different subtypes of γ -aminobutyric acid A (GABA_A) receptors, namely those containing the α_1 , α_2 , α_3 , or α_5 subunit, in addition to the γ_2 subunit (Sieghart, 2006). Genetic and pharmacological studies, by the means of the generation of mutant mouse lines [α_1 (H101R), α_2 (H101R), α_3 (H126R), and α_5 (H105R) knock-ins] (Rudolph and Möhler, 2004) and synthesis of novel, subtype-selective ligands, have helped in linking particular behavioral responses to specific GABA_A receptor subtypes. The sedative effects of BZs were principally attributed to the α_1 -GABA_A receptor subtype, anxiolytic actions to α_2/α_3 containing receptors, anterograde amnesic effects to α_1/α_5 subtypes, and anticonvulsant activity partially to α_1 -GABA_A receptors (Löw *et al.*, 2000; McKernan *et al.*, 2000; Collinson *et al.*, 2002; Savić *et al.*, 2009).

BZs negatively affect motor coordination and balance, that is, they induce ataxia, which is, together with myorelaxation, often referred to as motor impairment (Verster *et al.*, 2002; Licata *et al.*, 2009). In contrast to

ataxia, myorelaxation can be therapeutically desirable, and disentangling the molecular substrates of these two effects would benefit the development of compounds with an improved pharmacological profile. Like sedation, the impaired coordination and balance were also ascribed to potentiation at α_1 -GABA_A receptors and these results were consistent with experiments in both rodents and nonhuman primates (McKernan *et al.*, 2000; Platt *et al.*, 2002; Licata *et al.*, 2009). Ligands that lack or have substantially decreased activity at α_1 -GABA_A receptors, compared with conventional nonselective BZs, did not engender ataxia over the wide dose range tested (Licata *et al.*, 2005; Mirza *et al.*, 2008; Savić *et al.*, 2008; Atack 2010). The experiments on genetically modified mice have excluded the role of the α_1 subunit as a molecular substrate of myorelaxation (Rudolph *et al.*, 1999; McKernan *et al.*, 2000) and found that the myorelaxant properties of diazepam are mainly mediated by α_2 -GABA_A receptors; at very high doses of diazepam, the α_3 -GABA_A and α_5 -GABA_A receptor subtypes may also become implicated (Crestani *et al.*, 2001). However, a number of pharmacological studies have shown that muscle relaxation induced by nonselective BZ-site agonists could be reversed with the use of the α_1 -GABA_A selective antagonist β CCt, demonstrating ambiguity in this area (Griebel *et al.*, 1999; Licata *et al.*, 2009).

The overall aim of the present study was to examine, by pharmacological means, the extent to which α_1 -GABA_A and α_5 -GABA_A receptor subtypes contribute to BZ-induced ataxia and muscle relaxation in Wistar rats and to provide further information on the molecular substrates of these two effects. Benzodiazepine-induced ataxia in rodents is usually measured using the rotarod test (Mirza *et al.*, 2008; Savić *et al.*, 2008), whereas the myorelaxant effects of BZs are often assessed using the grip strength test (Maurissen *et al.*, 2003). In the present study, we used diazepam, a ligand with high efficacy and no selectivity for GABA_A receptor subtypes, and the α_1 -GABA_A receptor-selective agonist zolpidem, which possesses intermediate and no affinity for α_2/α_3 -GABA_A and α_5 -GABA_A receptor subtypes, respectively (Sanna *et al.*, 2002). Using the GABA_A nonselective antagonist flumazenil, the α_1 -subunit affinity-selective antagonist β CCt (Shannon *et al.*, 1984), and the α_5 -subunit affinity-selective and efficacy-selective antagonist XLi093 (Li *et al.*, 2003), we examined the degree to which zolpidem-induced and diazepam-induced ataxia and myorelaxation could be antagonized.

Methods

Subjects

Male Wistar rats, weighing 200–230 g, were supplied by Military Farm, Belgrade, Serbia. Rats were housed in groups of six and were maintained under standard laboratory conditions ($21 \pm 2^\circ\text{C}$, relative humidity 40–45%) with free access to pellet food and tap water. They were kept on a 12:12 h light/dark cycle with lights on at 07.00 h. All handling and testing took place during the light phase of the diurnal cycle. Experiments were carried out in accordance with the European Economic Community Directive 86/609 and were approved by the Ethical Committee on Animal Experimentation of the Faculty of Pharmacy in Belgrade.

Rotarod test

Motor performance was assessed using an automated rotarod (Ugo Basile, Comerio, Italy). Before testing, rats were trained for 3 days until they could remain on a revolving rod for 120 s with acceleration from 15 rpm to 25 rpm. During the training days, all animals were given three training sessions of 2 min each, with a 30 min intersession interval. On the fourth day, rats that met the given criteria were selected for inclusion in the experiment. Groups of 6–8 animals received one of the following treatments: diazepam (0 and 2 mg/kg) in combination with β CCt (0, 1, 5, 20, and 30 mg/kg), flumazenil (0, 10, and 20 mg/kg), or XLi093 (0, 10, and 20 mg/kg), as well as zolpidem (0, 1, 2, and 5 mg/kg) and zolpidem (0 and 2 mg/kg) combined with β CCt (0, 5, and 20 mg/kg) or flumazenil (0, 10, and 20 mg/kg). Latency to falling off the rod was recorded automatically for each animal.

Grip strength test

This test was used to examine the myorelaxant properties of agonists, antagonists, and their combinations. Two experiments were performed: in the first, animals received diazepam (0 and 2 mg/kg) in combination with three levels of flumazenil (0, 10, and 20 mg/kg), β CCt (0, 20, and 30 mg/kg), and XLi093 (0, 10 and 20 mg/kg); in the second experiment, animals received zolpidem (0, 1, 2, and 5 mg/kg) and zolpidem (0 and 5 mg/kg) in combination with β CCt (0 and 10 mg/kg). After administration of the appropriate treatment, rats were allowed to grip, with their front paws, a metal trapezoid wire attached to a grip-strength meter (Ugo Basile, Italy). Grip strength was tested by dragging the rat gently by the tail. The apparatus measured the pull force (expressed in grams) necessary to overcome the animal's forelimbs grip-strength to the bar connected to a force transducer. Each animal was given three consecutive trials and the maximum value was taken.

Drugs

The compounds used were diazepam (Galenika, Belgrade, Serbia), zolpidem (Toronto Chemical Research, Toronto, Ontario, Canada), flumazenil (Feicheng BoYuan Fine Chemicals Co. Ltd, East Feicheng, China), XLi093 (4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylic acid, 8-ethynyl-5,6-dihydro-5-methyl-6-oxo-, 1,3-propanediyl ester), the α_5 -subunit affinity-selective and efficacy-selective antagonist, and β CCt (*t*-butyl- β -carboline-3-carboxylate), the α_1 -subunit affinity-selective antagonist; the latter two agents were synthesized at the Department of Chemistry and Biochemistry, University of Wisconsin–Milwaukee, USA. The ligands were suspended in a solvent containing 85% distilled water, 14% propylene glycol, and 1% Tween-80. All animals received two intraperitoneal injections consisting of the appropriate ligand(s) and/or solvent (in a total volume of 2 ml/kg), 20 min before the testing. When a combination of two compounds was administered, the first compound was injected into the lower right and the second into the lower left quadrant of the peritoneum.

Statistics

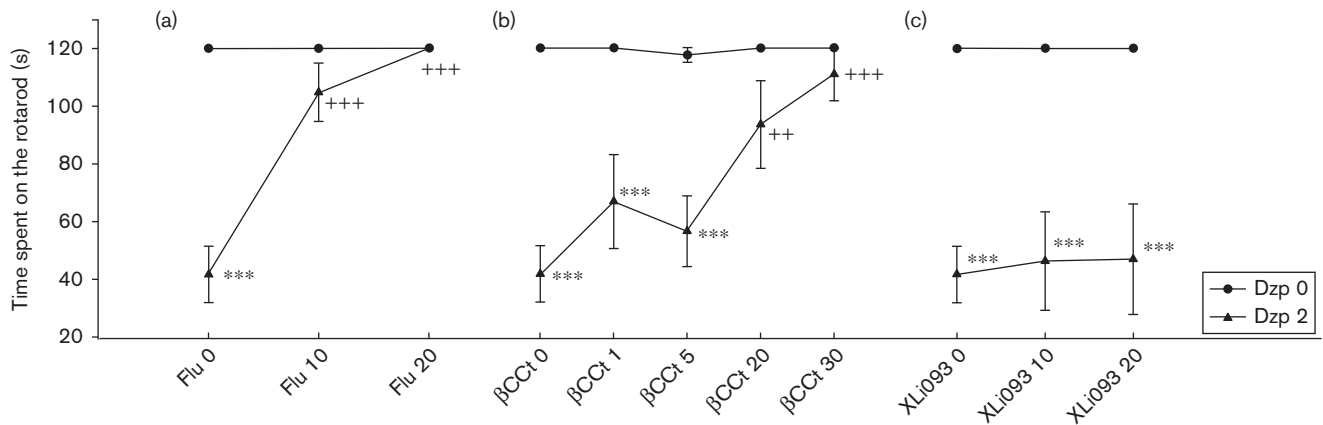
All numerical data presented in the figures are shown as the mean \pm SEM. The dose response of zolpidem was assessed using one-way analysis of variance (ANOVA), with a post-hoc Student–Newman–Keuls test. The effects of combined treatments were assessed using two-way ANOVA with a post-hoc Student–Newman–Keuls test, where applicable.

Results

Rotarod

Animals that received 2 mg/kg diazepam spent significantly less time on the rotarod than the control group of rats (Fig. 1; $P < 0.001$). When diazepam was injected immediately after flumazenil, a significant main effect of

Fig. 1



The influence of pretreatment with antagonists (a) flumazenil (Flu), (b) β CCt, and (c) XLI093 on diazepam-induced (Dzp) ataxia on the rotarod. Data are mean \pm SEM from $n=8$ rats per group. *** P less than 0.001 versus vehicle; ** P less than 0.01 versus 2 mg/kg diazepam; *** P less than 0.001 versus 2 mg/kg diazepam.

flumazenil [$F(2,40) = 18.07$, $P < 0.001$] and diazepam \times flumazenil interaction [$F(2,45) = 18.07$, $P < 0.001$] were found. Both 10 and 20 mg/kg of flumazenil antagonized the motor incoordination induced by diazepam (Fig. 1a; both $P < 0.001$ compared with 2 mg/kg diazepam). Similarly, coadministration of β CCt resulted in a significant treatment effect [$F(4,68) = 4.05$, $P < 0.005$] and a significant diazepam \times β CCt interaction [$F(4,77) = 3.83$, $P < 0.01$]. Although the two lower doses of β CCt (1 and 5 mg/kg) failed to antagonize the diazepam-induced motor impairment, coadministration of the two higher doses of β CCt (20 and 30 mg/kg) significantly increased the time spent on the rotarod (Fig. 1b; both $P < 0.001$), when compared with diazepam dosed at 2 mg/kg. XLI093, an α_5 -selective antagonist, did not antagonize the diazepam-induced motor incoordination (Fig. 1c).

All three doses of zolpidem (1, 2, and 5 mg/kg) impaired motor coordination (Fig. 2a; $P < 0.001$ in all three cases). Pretreatment with flumazenil significantly influenced the zolpidem-induced ataxia [zolpidem: $F(1,37) = 114.02$, $P < 0.001$; zolpidem \times flumazenil interaction: $F(2,42) = 108.54$, $P < 0.001$]. When compared with animals that received only 2 mg/kg of zolpidem, animals treated with the combination of zolpidem 2 mg/kg + flumazenil (10 or 20 mg/kg) spent significantly more time on the rotarod (Fig. 2b; $P < 0.001$ and $P < 0.001$, respectively). The effect on motor coordination of β CCt [$F(1,34) = 73.94$, $P < 0.001$] and the zolpidem \times β CCt interaction [$F(2,39) = 40.61$, $P < 0.001$] was also significant. The subsequent post-hoc test showed that both 5 and 20 mg/kg of β CCt antagonized the zolpidem-induced ataxia (Fig. 2c; both $P < 0.001$, compared with 2 mg/kg zolpidem). There was also a significant difference in the time spent on the rotarod between animals that received 2 mg/kg zolpidem + 5 mg/kg β CCt and animals that received only 5 mg/kg β CCt

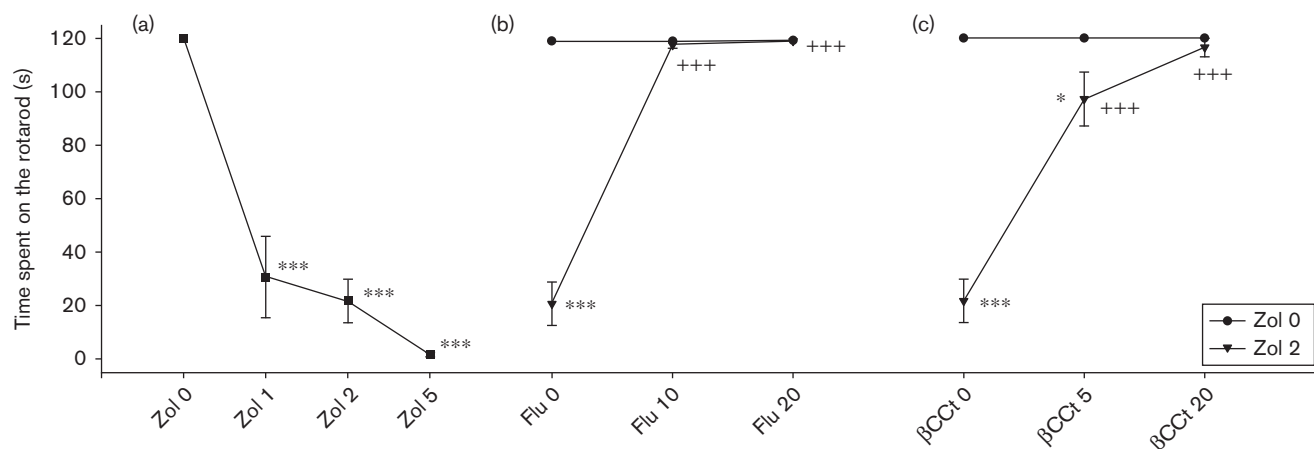
($P < 0.025$). None of the antagonists (flumazenil, β CCt, and XLI093) itself impaired the motor performance on the rotarod.

Grip strength

Application of 2 mg/kg diazepam produced significant muscle relaxation (Fig. 3; $P < 0.01$, relative to control). For the combination of diazepam + flumazenil, two-way ANOVA showed significant effects of diazepam [$F(1,31) = 6.09$, $P < 0.02$] and the flumazenil \times diazepam interaction [$F(2,36) = 5.94$, $P < 0.01$]; coadministration of flumazenil (10 and 20 mg/kg) reversed the diazepam-induced myorelaxation (Fig. 3a; $P < 0.001$ and $P < 0.01$, compared with diazepam 2 mg/kg, respectively). As with flumazenil, the effect of β CCt did not reach statistical significance, whereas the effect of diazepam [$F(1,28) = 7.82$, $P < 0.01$] as well as the interaction [$F(2,33) = 5.83$, $P < 0.01$] were significant. There were significant differences between the group that received 2 mg/kg diazepam and groups that received 2 mg/kg diazepam with either 20 or 30 mg/kg of β CCt (Fig. 3b; $P < 0.05$ and $P < 0.001$, respectively). The assessment of the results obtained with the α_5 -selective antagonist showed no significant effect of XLI093 on grip strength [$F(2,30) = 2.46$, NS], but showed a significant diazepam \times XLI093 interaction [$F(2, 35) = 6.18$, $P < 0.01$]; the differences between groups that received diazepam + XLI093 (10 and 20 mg/kg) and the group that received diazepam were statistically significant (Fig. 3c; $P < 0.002$ and $P < 0.005$, respectively).

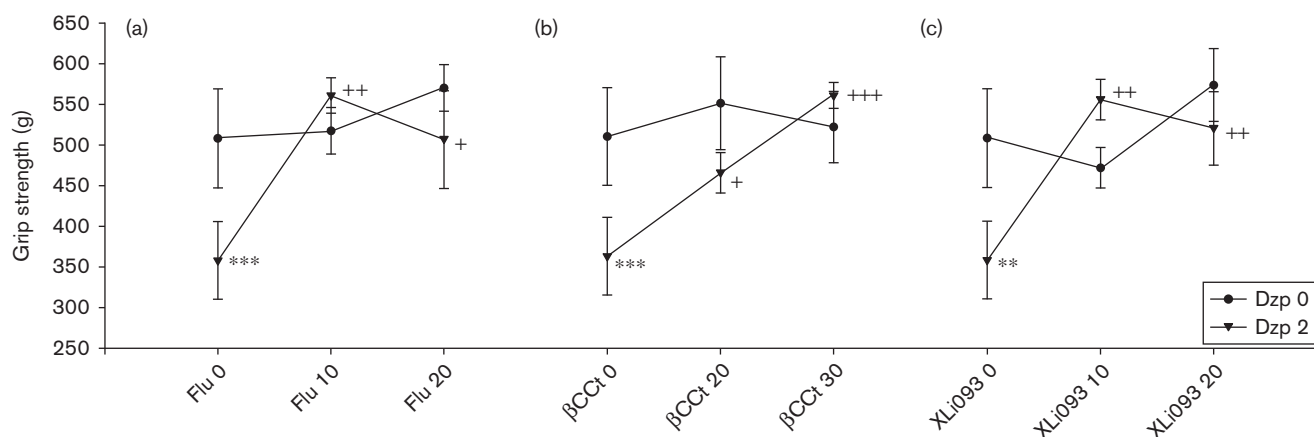
Zolpidem significantly decreased grip strength [$F(3,20) = 10.34$, $P < 0.001$]. Muscle relaxation was significant with 5 mg/kg zolpidem ($P < 0.001$), whereas the two lower doses (1 and 2 mg/kg) were at the control level (Fig. 4a). When the combination 5 mg/kg zolpidem + 10 mg/kg β CCt was assessed, significant effects of

Fig. 2



(a) Effects of zolpidem (Zol) on rotarod performance and the influence of pretreatment with (b) flumazenil (Flu) and (c) β CCt on ataxia induced by zolpidem (2 mg/kg). Data are mean \pm SEM from $n=6$ rats per group. * P less than 0.05 versus vehicle; *** P less than 0.001 versus vehicle; +++ P less than 0.001 versus 2 mg/kg zolpidem.

Fig. 3



The influence of pretreatment with the antagonists (a) flumazenil (Flu), (b) β CCt, and (c) XLI093 on the diazepam-induced (Dzp) muscle relaxation measured in the grip strength test. Data are mean \pm SEM from $n=8$ rats per group. ** P less than 0.05 versus vehicle; *** P less than 0.001 versus vehicle; + P less than 0.05 versus 2 mg/kg diazepam; ++ P less than 0.01 versus 2 mg/kg diazepam; +++ P less than 0.001 versus 2 mg/kg diazepam.

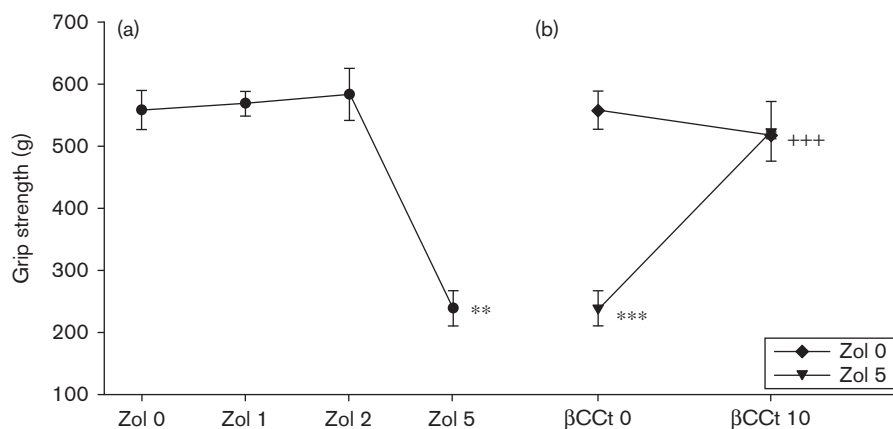
zolpidem [$F(1,15) = 19.74$, $P < 0.001$], β CCt [$F(1,15) = 16.11$, $P < 0.001$] and their interaction [$F(1,18) = 27.53$, $P < 0.001$] were found. Although β CCt itself did not alter grip strength, its addition to zolpidem reversed the zolpidem-induced muscle relaxation (Fig. 4b; $P < 0.001$, compared with 5 mg/kg zolpidem).

Discussion

Studies on genetically modified mice, in which a distinct α subunit of GABA_A receptors is rendered insensitive to diazepam, represent valuable tools in revealing which receptor subtype is necessary for the expression of a specific behavioral response. These experiments pointed

toward α_1 -GABA_A receptors as the main subtype in eliciting ataxia in mice (McKernan *et al.*, 2000). In the present study, diazepam-induced and zolpidem-induced ataxia on the rotarod in rats was successfully antagonized with the α_1 -selective antagonist β CCt. Because of its 20-fold selectivity for α_1 -GABA_A receptors compared with α_2 -GABA_A and α_3 -GABA_A receptors, β CCt is one of the most selective BZ-site ligands identified to date (Cox *et al.*, 1995; Huang *et al.*, 2000). In many behavioral studies, β CCt successfully reversed the effects of BZs related to the α_1 -GABA_A receptor subtype, such as ataxia, sedation, and anticonvulsant activity (Griebel *et al.*, 1999; Platt *et al.*, 2002; Savić *et al.*, 2009). However, not

Fig. 4



(a) Muscle-relaxant effect of zolpidem (Zol) and (b) the influence of pretreatment with β CCt. Data are mean \pm SEM from $n=6$ rats per group. ** P less than 0.05 versus vehicle; *** P less than 0.001 versus vehicle; +++ P less than 0.001 versus 5 mg/kg zolpidem.

all experiments using β CCt as the α_1 -selective ligand have reported antagonism of the diazepam-induced ataxia in mice or rats. Such discrepancies may have resulted from differences in the experimental design. Shannon and colleagues, (1984) reported that administration of 30 mg/kg β CCt did not attenuate the diazepam-induced ataxia in mice. The degree of motor impairment was assessed using an inverted-screen test, where the concomitant myorelaxation was likely to influence the performance of the test. Another study found that motor incoordination engendered by diazepam, triazolam, and zolpidem in mouse pups was not sensitive to β CCt (Rowlett *et al.*, 2001). However, motor impairment was related to rolling motions, as opposed to normal locomotor activity of mouse pups, and probably involved a predominantly spinal mechanism and engagement of α_2 -GABA_A and α_3 -GABA_A receptor subtypes (McKernan and Whiting, 1996). In the present study, the dose of β CCt needed to antagonize zolpidem-induced ataxia was substantially lower than the dose that antagonized the effect of diazepam (5 vs. 20 mg/kg). This implies that an effect of diazepam, possibly myorelaxation, mediated by receptors other than the α_1 -GABA_A receptor, may have contributed to the influence of diazepam, but not zolpidem, on rotarod test performance. In this scenario, the dose of 20 mg/kg of β CCt may have either blocked the α_1 -GABA_A receptor population more completely or started to prevent binding of diazepam to non- α_1 -GABA_A receptors.

The possibility that the α_5 -GABA_A receptor subtype plays a modulatory role in behavioral effects predominantly conferred by the α_1 subunit, such as sedation, tolerance development, and memory impairment, has been previously proposed (van Rijnsoever *et al.*, 2004; Savić *et al.*, 2008; Savić *et al.*, 2009). Hence, we tested the ability of the α_5 selective antagonist XLI093 to influence the

diazepam-induced ataxia. At the dose of 20 mg/kg, which was previously shown to intensify diazepam-induced sedation (Savić *et al.*, 2009), XLI093 did not significantly affect the motor-impairing effect of diazepam. This means that ataxia, as assessed in the rotarod test in rats, is not dependent on the activation of α_5 -GABA_A receptors.

Although genetic studies did not detect any role of the α_1 subunit in mediating muscle relaxation (Rudolph *et al.*, 1999; McKernan *et al.*, 2000), the data from experiments with subtype-selective ligands varied from one study to another depending on the species used and the dose of agonist or antagonist applied (Griebel *et al.*, 1999; Elliot and White, 2001; Licata *et al.*, 2009). In a radiotelemetric study in rats, zolpidem at the dose of 5 mg/kg, but not 2.5 mg/kg, induced a significant decrease in electromyographic activity, a parameter aimed to assess muscle relaxation (Elliot and White, 2001). In the present study, significant myorelaxation observed after both diazepam and zolpidem administration was prevented by pretreatment with β CCt. As the dose of zolpidem producing myorelaxation (5 mg/kg) was substantially higher than the minimal dose that induced ataxia (1 mg/kg), the possibility that zolpidem-induced myorelaxation is not mediated by α_1 -GABA_A receptors needs to be discussed. Despite its binding preference for α_1 -GABA_A receptors, zolpidem also binds to and potentiates effects at α_2 -GABA_A and α_3 -GABA_A receptors (Sanna *et al.*, 2002). The in-vivo selectivity of zolpidem for the α_1 -enriched cerebellum, in contrast to α_2/α_3 -enriched spinal cord, assessed through the reduction in flumazenil binding, is generally less than the α_1 selectivity of this compound *in vitro* (Atack *et al.*, 1999). However, the displacement curve for zolpidem in the spinal cord of rats (Benavides *et al.*, 1992) and mice (Atack *et al.*, 1999) is relatively flat, and very high doses of zolpidem (> 30 mg/kg in mice; Atack *et al.*, 1999) are

needed for half-inhibition of radio-labeled flumazenil binding in this region predominantly implicated in GABA-mediated myorelaxation (Bohlhalter *et al.*, 1996). Thus, one can conclude that muscle-relaxant effect of zolpidem at the dose of 5 mg/kg may not be exclusively mediated by α_2 -GABA_A receptors, the subtype largely responsible for the muscle-relaxant effect of diazepam (Crestani *et al.*, 2001). In contrast, β CCt (30 mg/kg) reversed diazepam-induced muscle relaxation in mice (Griebel *et al.*, 1999), and at the dose of 3 mg/kg, it attenuated the myorelaxant properties of several nonselective benzodiazepine agonists in squirrel monkeys (Licata *et al.*, 2009). The propensity of β CCt to antagonize some of the principally non- α_1 -mediated effects of diazepam was also shown in the elevated plus-maze and light-dark test of anxiety (Griebel *et al.*, 1999; Belzung *et al.*, 2000). Nonetheless, a potentiating effect of 30 mg/kg β CCt on the anxiolytic actions of BZs in rats has also been repeatedly reported (Savić *et al.*, 2004, 2005), which cannot be a consequence of putative antagonism on α_2 -GABA_A receptors. Assessment of the ability of 10 mg/kg β CCt (intraperitoneally) to displace the radio-labeled flumazenil in mice indicates that β CCt at the given dose level preferentially targets the cerebellum, whereas it binds to less than 40% of GABA_A receptors, mainly of the α_2 -subtype, in the spinal cord (Rowlett *et al.*, 2005). Given the doses of zolpidem and β CCt that we used, we hypothesize that under our experimental conditions, the actions of these ligands may, to a small extent, have involved the α_2 -GABA_A receptor subtype, in addition to the predominantly affected α_1 -GABA_A receptor subtype. In the presence of intense activation of α_1 -GABA_A receptors by a large dose of zolpidem, the presumed small involvement of α_2 -GABA_A receptors may have been large enough to trigger muscle relaxation.

The contribution of the α_5 subunit in mediating the muscle-relaxant effect of diazepam was observed in α_5 (H105R) mutant mice (Crestani *et al.*, 2002). Here, we report on antagonism of the muscle-relaxant effect of diazepam with the α_5 -selective ligand XLi093 in rats. Nonetheless, muscle relaxation can be achieved without the apparent activation of α_5 -GABA_A receptors, as demonstrated in experiments with zolpidem (Elliot and White, 2001; Licata *et al.*, 2009). Furthermore, an α_2/α_3 selective compound devoid of agonistic activity at the α_5 subunit exerted muscle relaxation in monkeys (Licata *et al.*, 2005). These results suggest that the role of the α_5 subunit in the BZ-induced myorelaxation could be described as nondominant, but still significant, and should be further investigated.

The present study demonstrates that α_1 -GABA_A and α_5 -GABA_A receptor subtypes differentially contribute to the motor-impairing effects of BZs in rats. Although activation of α_1 -GABA_A receptors is a prerequisite for eliciting ataxia, these receptors are probably not directly involved in mediating muscle relaxation but still may

contribute to the manifestation of this effect triggered by a small fraction of activated α_2 -GABA_A receptors. In contrast, activation of α_5 -GABA_A receptors contributes significantly, although not dominantly, to muscle relaxation, but not ataxia. Thus, in the quest for ligands with an improved pharmacological profile, it could be of importance to avoid substantial potentiation through α_1 subunits, if ataxia is to be prevented, whereas a certain level of activation at both α_1 and α_5 subunits could be advantageous when muscle relaxation is required.

Acknowledgements

The authors acknowledge the support by The Ministry of Science, R. Serbia – Grant No. 175076 (MMS) and by NIMH grant MH-046851 (JMC).

Conflicts of interest

There are no conflicts of interest.

References

- Attack JR (2010). GABAA receptor alpha2/alpha3 subtype-selective modulators as potential nonsedating anxiolytics. *Curr Top Behav Neurosci* 2: 331–360.
- Attack JR, Smith AJ, Emms F, McKernan RM (1999). Regional differences in the inhibition of mouse in vivo [³H]Ro 15-1788 binding reflect selectivity for alpha 1 versus alpha 2 and alpha 3 subunit-containing GABAA receptors. *Neuropsychopharmacology* 20:255–262.
- Belzung C, Le Guesquet AM, Griebel G (2000). Beta-CCT, a selective BZ-omega1 receptor antagonist, blocks the anti-anxiety but not the amnesic action of chlordiazepoxide in mice. *Behav Pharmacol* 11:125–131.
- Benavides J, Peny B, Durand A, Arbilla S, Scatton B (1992). Comparative in vivo and in vitro regional selectivity of central omega (benzodiazepine) site ligands in inhibiting [³H]flumazenil binding in the rat central nervous system. *J Pharmacol Exp Ther* 263:884–896.
- Bohlhalter S, Weinmann O, Mohler H, Fritschy JM (1996). Laminar compartmentalization of GABAA-receptor subtypes in the spinal cord: an immunohistochemical study. *J Neurosci* 16:283–297.
- Cox ED, Hagen TJ, McKernan RM, Cook JM (1995). BZ1 receptor subtype specific ligands. Synthesis and biological properties of beta-CCT, a BZ1 receptor subtype specific antagonist. *Med Chem Res* 5:710–718.
- Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, *et al.* (2002). Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. *J Neurosci* 22:5572–5580.
- Crestani F, Löw K, Keist R, Mandelli M, Möhler H, Rudolph U (2001). Molecular targets for the myorelaxant action of diazepam. *Mol Pharmacol* 59:442–445.
- Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, *et al.* (2002). Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. *Proc Natl Acad Sci USA* 99:8980–8985.
- Elliot EE, White JM (2001). The acute effects of zolpidem compared to diazepam and lorazepam using radiotelemetry. *Neuropharmacology* 40:717–721.
- Griebel G, Perrault G, Letang V, Granger P, Avenet P, Schoemaker H, Sanger DJ (1999). New evidence that the pharmacological effects of benzodiazepine receptor ligands can be associated with activities at different BZ (omega) receptor subtypes. *Psychopharmacology (Berl)* 146:205–213.
- Huang Q, He X, Ma C, Liu R, Yu S, Dayer CA, *et al.* (2000). Pharmacophore/receptor models for GABA(A)/BzR subtypes (alpha1beta3gamma2, alpha5-beta3gamma2, and alpha6beta3gamma2) via a comprehensive ligand-mapping approach. *J Med Chem* 13:71–95.
- Li X, Cao H, Zhang C, Furtmueller R, Fuchs K, Huck S, *et al.* (2003). Synthesis, in vitro affinity, and efficacy of a bis 8-ethynyl-4H-imidazo[1,5a]-[1,4]benzodiazepine analogue, the first bivalent alpha5 subtype selective BzR/GABA(A) antagonist. *J Med Chem* 46:5567–5570.
- Licata SC, Platt DM, Cook JM, Sarma PV, Griebel G, Rowlett JK (2005). Contribution of GABAA receptor subtypes to the anxiolytic-like, motor, and discriminative stimulus effects of benzodiazepines: studies with the functionally selective ligand SL651498 [6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyridol[3,4-b]indol-1-one]. *J Pharmacol Exp Ther* 313:1118–1125.

- Licata SC, Platt DM, Cook JM, van Linn ML, Rowlett JK (2009). Contribution of alpha1 subunit-containing gamma-aminobutyric acidA (GABAA) receptors to motor-impairing effects of benzodiazepines in squirrel monkeys. *Psychopharmacology (Berl)* **203**:539–546.
- Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson JA, *et al.* (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* **290**:131–134.
- Maurissen JP, Marable BR, Andrus AK, Stebbins KE (2003). Factors affecting grip strength testing. *Neurotoxicol Teratol* **25**:543–553.
- McKernan RM, Whiting PJ (1996). Which GABAA-receptor subtypes really occur in the brain? *Trends Neurosci* **19**:139–143.
- McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, *et al.* (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. *Nat Neurosci* **6**:587–592.
- Mirza NR, Larsen JS, Mathiasen C, Jacobsen TA, Munro G, Erichsen HK, *et al.* (2008). NS11394 [3'-[5-(1-hydroxy-1-methyl-ethyl)-benzimidazol-1-yl]-bi-phenyl-2-carbonitrile], a unique subtype-selective GABAA receptor positive allosteric modulator: in vitro actions, pharmacokinetic properties and in vivo anxiolytic efficacy. *J Pharmacol Exp Ther* **327**:954–968.
- Platt DM, Rowlett JK, Spealman RD, Cook J, Ma C (2002). Selective antagonism of the ataxic effects of zolpidem and triazolam by the GABAA/alpha1-preferring antagonist beta-CCt in squirrel monkeys. *Psychopharmacology (Berl)* **164**:151–159.
- Rowlett JK, Tornatzky W, Cook JM, Ma C, Miczek KA (2001). Zolpidem, triazolam, and diazepam decrease distress vocalizations in mouse pups: differential antagonism by flumazenil and beta-Carboline-3-carboxylate-t-butyl ester (beta-CCt). *J Pharmacol Exp Ther* **297**:247–253.
- Rowlett JK, Cook JM, Duke AN, Platt DM (2005). Selective antagonism of GABAA receptor subtypes: an in vivo approach to exploring the therapeutic and side effects of benzodiazepine-type drugs. *CNS Spectr* **10**:40–48.
- Rudolph U, Möhler H (2004). Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics (Review). *Annu Rev Pharmacol Toxicol* **44**:475–498.
- Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy JM, *et al.* (1999). Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature* **401**:796–800.
- Sanna E, Busonero F, Talani G, Carta M, Massa F, Peis M, *et al.* (2002). Comparison of the effects of zaleplon, zolpidem, and triazolam at various GABA(A) receptor subtypes. *Eur J Pharmacol* **451**:103–110.
- Savić MM, Obradović DI, Ugresić ND, Cook JM, Yin W, Bokonić DR (2004). Bidirectional effects of benzodiazepine binding site ligands in the elevated plus-maze: differential antagonism by flumazenil and beta-CCt. *Pharmacol Biochem Behav* **79**:279–290.
- Savić MM, Obradović DI, Ugresić ND, Cook JM, Sarma PV, Bokonić DR (2005). Bidirectional effects of benzodiazepine binding site ligands on active avoidance acquisition and retention: differential antagonism by flumazenil and beta-CCt. *Psychopharmacology (Berl)* **180**:455–465.
- Savić MM, Huang S, Furtmüller R, Clayton T, Huck S, Obradović DI, *et al.* (2008). Are GABAA receptors containing alpha5 subunits contributing to the sedative properties of benzodiazepine site agonists? *Neuropsychopharmacology* **33**:332–339.
- Savić MM, Milinković MM, Rallapalli S, Clayton T Sr, Joksimović S, Van Linn M, Cook JM (2009). The differential role of alpha1- and alpha5-containing GABA(A) receptors in mediating diazepam effects on spontaneous locomotor activity and water-maze learning and memory in rats. *Int J Neuropsychopharmacol* **12**:1179–1193.
- Shannon HE, Guzman F, Cook JM (1984). Beta-Carboline-3-carboxylate-t-butyl ester: a selective BZ1 benzodiazepine receptor antagonist. *Life Sci* **35**:2227–2236.
- Sieghart W (2006). Structure, pharmacology, and function of GABAA receptor subtypes. *Adv Pharmacol* **54**:231–263.
- Van Rijnsoever C, Täuber M, Choulli MK, Keist R, Rudolph U, Mohler H, *et al.* (2004). Requirement of alpha5-GABAA receptors for the development of tolerance to the sedative action of diazepam in mice. *J Neurosci* **24**:6785–6790.
- Verster JC, Volkerts ER, Verbaten MN (2002). Effects of alprazolam on driving ability, memory functioning and psychomotor performance: a randomized, placebo-controlled study. *Neuropsychopharmacology* **27**:260–269.