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Benzodiazepine site inverse agonists and locomotor activity in rats: Bimodal and biphasic influence

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Abstract

Benzodiazepine site inverse agonists may increase or decrease locomotor activity in rodents, depending on the experimental settings. We have compared the behavioral responses to environmental novelty of rats treated with the non-selective inverse agonist DMCM (2 mg/kg) and the α_1-subunit affinity-selective inverse agonist 3-EBC (15 mg/kg). The behavior in spontaneous locomotor assay (during 45 min) and elevated plus maze (EPM) was automatically recorded. In the EPM, general activity-related parameters were similarly decreased, whereas only DMCM inhibited open-arm activity. In the locomotor assay, both compounds depressed locomotion in the first 15 min and activity in the central zone of the chamber. However, the influence of 3-EBC was less pronounced. The α_1-subunit selective antagonist β-CCt (15 mg/kg) attenuated locomotor depression, but not the central-zone avoidance elicited by DMCM. When habituated to the chamber, DMCM-treated animals emitted a plateau phase of activity, which disappeared by adding β-CCt. Hence, inhibition of activity in exposed areas may be mediated by non-α_1-subunits, whereas both α_1 and non-α_1-subunits may participate in suppression of activity in more protective areas of an apparatus. Hyperlocomotion in habituated animals may depend primarily on the α_1-subunit. Moreover, the bimodal influence of inverse agonists on locomotion can be biphasic, observable in the same experiment.

Keywords: Anxiety; Exploration; β-carbolines; $GABA_{A1}$ receptor; Elevated plus maze; Spontaneous locomotor activity

1. Introduction

Studies of motor activity form the very basis of knowledge of drug effects on behavior and comprise the analysis of changes in general motor activity and exploration (Kelley, 1993). Exploration refers to behaviors that are triggered off by novelty (Belzung, 1999). When exposed to novel environments, such as an activity monitor or a maze, animals exhibit both curiosity and fear (Kelley, 1993). It is exceptionally difficult to dissociate between motivational (curiosity-related) and emotional (neophobia-related) effects in tests of exploratory behavior (Kelley, 1993; Otter et al., 1997). On the other side, when an animal is habituated to the enclosure, the exploratory drive is decreased and the emitted behavior may rather reflect general, spontaneous locomotor activity (Kelley, 1993). Besides, the exploratory behavior and spontaneous locomotor activity are sometimes recorded as separate tests in the same apparatus, under different conditions (Voikar et al., 2001).

Ligands at the benzodiazepine binding site of the $GABA_A$ receptor are among the behaviorally most studied drugs. There are three kinds of allosteric modulators acting through this binding site: positive (agonist), neutral (antagonist), and negative (inverse agonist) modulators (Chebib and Johnston, 2000). Agonists and inverse agonists commonly exert bidirectional influences on observed behavioral parameters (Chapouthier and Venault, 2002; Jensen et al., 1987; Pellow and File, 1986; Savić et al., 2004, 2005a,b; Stephens et al., 1987). As regards motor activity, the inverse agonists, most notably β-carboline compounds, may increase or decrease locomotion in rodents, depending on the

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Table 1
Binding affinity (Ki, nM) at human recombinant GABAA receptors containing β3, γ_2 and named α-subunit, stably expressed in mouse fibroblast L(tk$^-$) cells (Huang et al., 2000)

	α1	α2	α3	α5
DMCM	5.7	8.3	4.0	1.04
3-EBC	6.43	25.1	28.2	826
β-CCt	0.72	15.0	18.9	111

experimental settings. As a rule, the stimulant effects may be seen in a familiar context (Crestani et al., 2002; Jackson and Nutt, 1992), whereas the depressant influence could be expected when the animal explores a novel environment (Jaskiw et al., 2003; Novas et al., 1988; Nagatani et al., 1990; Otter et al., 1997; Pahkla et al., 2000).

The vast majority of $GABA_A$ receptors appear to be associations of two α-subunits, two β-subunits and a single γ-subunit, which comprise a central ion channel (Chang et al., 1996). The majority of them contain a benzodiazepine binding site located at the interface of the γ_2-subunit and the respective α-subunit (α_1, α_2, α_3 or α_5) (Sigel and Buhr, 1997). Recent genetic and pharmacological studies pointed to the specific contribution of individual receptor subtypes to the spectrum of behavioral actions of benzodiazepine site ligands. Specifically, sedative effects of benzodiazepines were mainly attributed to α_1-containing $GABA_A$ receptor subtypes, anxiolytic action to the α_2/α_3-containing receptors, anterograde amnesic effects to the α_1/α_5-subtypes, anticonvulsant activity partially to the α_1-containing receptors, and muscle relaxant effect largely to the α_2-containing receptors (Atack et al., 2006; Low et al., 2000; McKernan et al., 2000; Rudolph and Mohler, 2006; Rudolph et al., 1999).

On the other hand, anxiogenic properties of inverse agonists were attributed to α_3-containing subtypes (Atack et al., 2005), and promnesic effects mainly to α_5-containing receptors (Sternfeld et al., 2004; Dawson et al., 2006). As regards the α_1-subtype, a set of results on mice with a single histidine to arginine point mutation (Wieland et al., 1992) suggested that proconvulsive action of inverse agonists, as well as motor stimulation in habituated animals, may depend on this subunit (Crestani et al., 2002); however, studies with α_1-subtype selective ligands, which are needed to corroborate insights provided by genetic studies, are still lacking.

In the present study, we have compared the modulation of behavioral responses to environmental novelty in two unconditioned paradigms: spontaneous locomotor activity and elevated plus maze, elicited by two β-carboline inverse agonists. DMCM (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) is a non-selective inverse agonist (DMCM), whereas 3-EBC (3-ethoxy-β-carboline-3-carboxylate) is a preferential α_1-subunit affinity-selective inverse agonist (Allen et al., 1988; Trullas et al., 1988; Huang et al., 2000). The influence of the preferential α_1-subunit selective antagonist β-CCt (*t*-butyl-β-carboline-3-carboxylate) (Cox et al., 1995) on the effects of inverse agonists was also examined. Binding affinities at recombinant $GABA_A$ receptors of ligands used are given in Table 1. Possible confounding influence on motor execution was checked by determination of median incapacitating doses of these β-carbolines in a rotarod test. The aim of the study was to assess if the locomotor response of rats to novelty is affected differently by DMCM and 3-EBC and, in the case of an affirmative answer, to suggest the relative significance of the α_1-subunit in motor effects of inverse agonists.

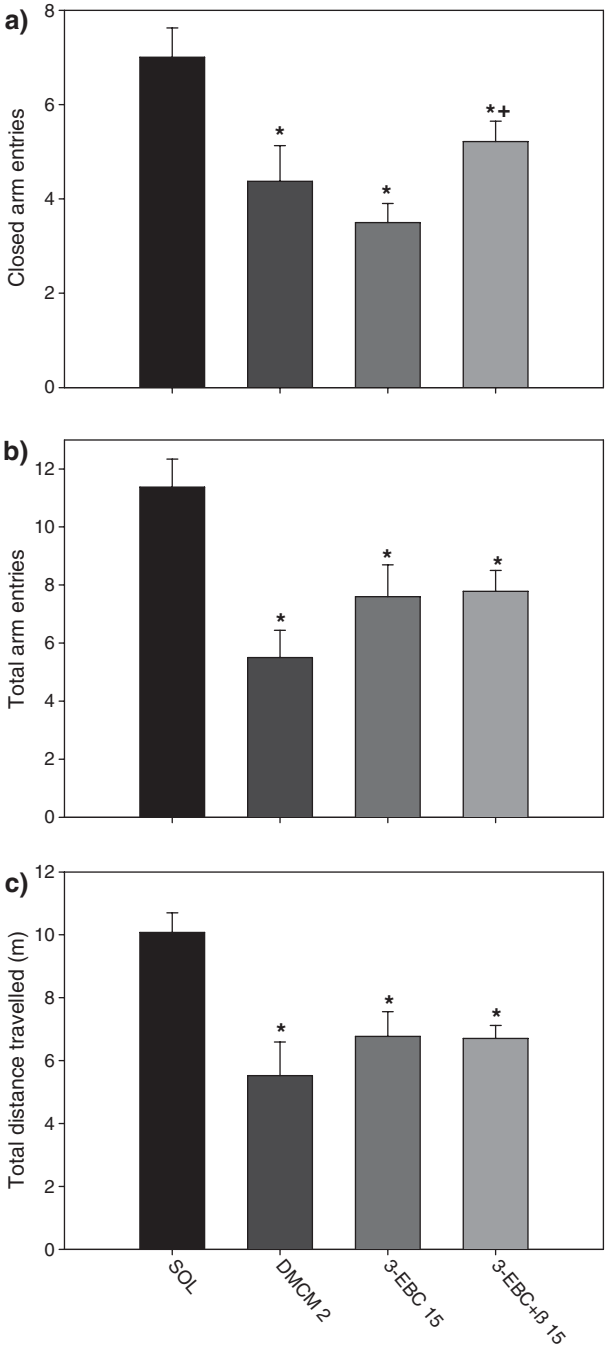

Fig. 1. The effects of DMCM (2 mg/kg), 3-EBC (15 mg/kg) and combination of 3-EBC (15 mg/kg)+β-CCt (β: 15 mg/kg) on the a) closed arm entries, b) total arm entries and c) total distance travelled in the EPM. *$p<0.05$ compared to solvent (SOL) group. $^+p<0.05$ compared to 3-EBC group. Number of animals per treatment (Figs. 1 and 2, for SOL through 3-EBC+β15, respectively): 8, 8, 10, 14.

2. Materials and methods

2.1. Animals

Experiments were carried out on male Wistar rats (Military Farm, Belgrade, Serbia and Montenegro), weighing 200–240 g. All procedures in the study conformed to EEC Directive 86/609 and were approved by the Ethical Committee on Animal Experimentation of the Medical Faculty in Belgrade. The rats were housed in transparent plastic cages, six animals per cage, and had free access to pelleted food and tap water. The temperature of the animal room was 22±1 °C, the relative humidity 40–70%, the illumination 120 lux, and the 12/12 h light/dark period (light on at 6:00 h). All handling and testing took place during the light portion of the cycle. Throughout the study the animals were used only once.

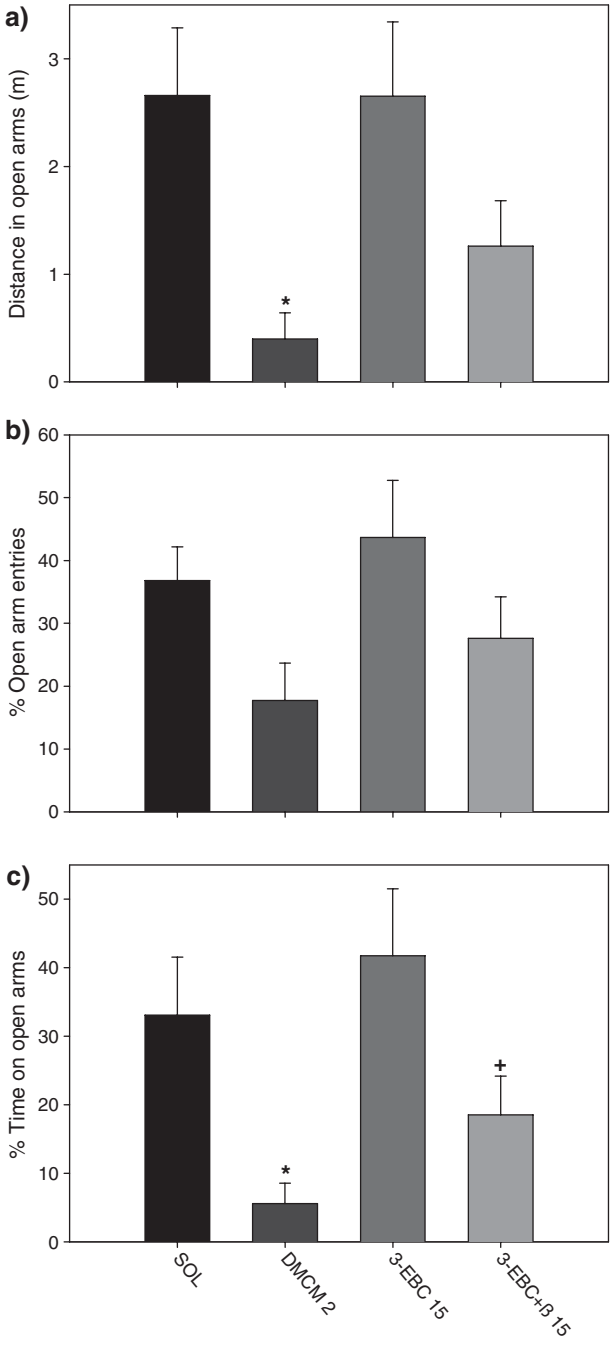

Fig. 2. The effects of DMCM (2 mg/kg), 3-EBC (15 mg/kg) and combination of 3-EBC (15 mg/kg)+β-CCt (β: 15 mg/kg) on the a) distance travelled on open arms, b) percentage of entries in open arms and c) percentage of time spent on open arms of the EPM. *$p<0.05$ compared to solvent (SOL) group; $^{+}p<0.05$ compared to 3-EBC group.

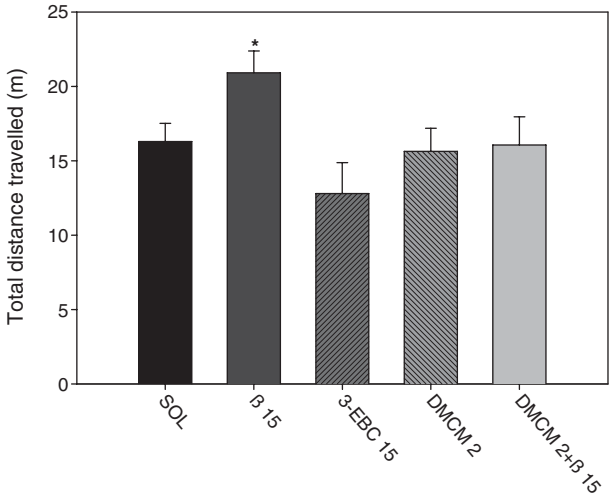

Fig. 3. The effects of β-CCt (β: 15 mg/kg), 3-EBC (15 mg/kg), DMCM (2 mg/kg), and combination of DMCM (2 mg/kg)+β-CCt (β: 15 mg/kg) on the total distance travelled in the activity assay. *$p<0.05$ compared to solvent (SOL) group. Number of animals per treatment (Figs. 3–5, for SOL through DMCM 2+β15, respectively): 9, 9, 10, 10, 6.

2.2. Drugs

DMCM was purchased from Research Biochemicals Incorporated (Natick, MA, USA). 3-EBC and β-CCt were synthesized as described in detail previously (Allen et al., 1988; Cox et al., 1995). All drugs were dissolved/suspended with the aid of sonication in a solvent containing 85% distilled water, 14% propylene glycol, and 1% Tween 80, and were administered intraperitoneally, in a volume of 1 ml/kg, 20 min before testing. The doses were chosen according to the previous results in paradigms of unconditioned behavior (June et al., 2003; Savić et al., 2004; Trullas et al., 1988). In the cases of combined treatment, inverse agonists were administered at separate sites, immediately after the antagonist. Each animal received a total volume of 2 ml/kg of compounds tested or solvent, at two different injection sites. Throughout the study, the number of rats per treatment group was 6–14 (indicated in the legends of Figs. 1 and 3).

2.3. Behavior on the elevated plus maze

The apparatus was constructed of sheet metal, with a black rubber floor. It consisted of a maze elevated to a height of 50 cm with two open (50×10 cm) and two enclosed arms (50×10×40 cm), connected by a junction area (central platform) measured 10×10 cm. A ledge of sheet metal (0.3 cm high) surrounding the open arms was added. In order to decrease

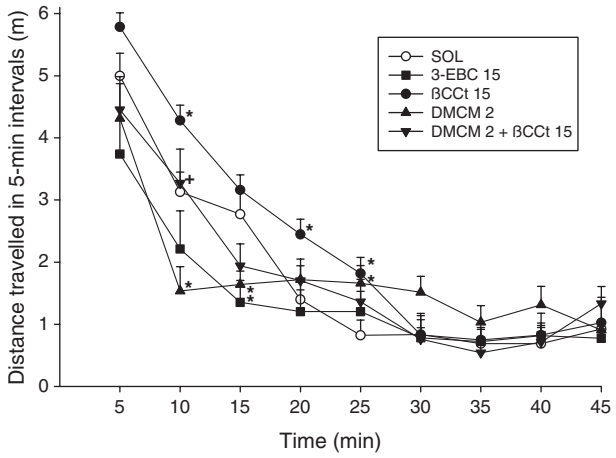

Fig. 4. The effects of β-CCt (15 mg/kg), 3-EBC (15 mg/kg), DMCM (2 mg/kg), and combination of DMCM (2 mg/kg)+β-CCt (β: 15 mg/kg) on the distance travelled in 5-min intervals in the activity assay. *$p<0.05$ compared to solvent (SOL) group; $^{+}p<0.05$ compared to DMCM group.

baseline avoidance of the open arms (cf. Savić et al., 2004), the illumination in the experimental room consisted of one red neon tube fixed on the ceiling, giving light intensity of 10 lux on the surface of the arms.

The experiments were carried out during the diurnal phase (between 08:00 and 14:00 h). At the beginning of the experiment, rats were placed in the center of the maze, facing one of the enclosed arms, and their behavior was recorded for 5 min. A digital camera was mounted above the maze and the image was transmitted to a notebook computer in the neighboring room, running the ANY-maze Video Tracking System software (Stoelting Co., Wood Dale, IL, USA). An entry into an open or closed arm was scored when 90% of the animal crosses the virtual line separating the central square of the maze from the arm, whereas an exit occurred when 10% of the animal leaves the respective arm. After each trial, the maze was cleaned with dry and wet towels.

The effects of DMCM (2 mg/kg) and 3-EBC (15 mg/kg) on behavior in the EPM were evaluated, as well as the influence of β-CCt (15 mg/kg) on the effects of 3-EBC (15 mg/kg).

2.4. Measurement of locomotor activity

Immediately after receiving the appropriate treatment, single rats were placed in a clear Plexiglas chamber (40×25×35 cm). Activity under dim red light (20 lux) was recorded for a total of 45 min, without any habituation period, using ANY-maze software (as described above). For purposes of improving data analysis, the central 20% of the chamber (200 cm^2) was virtually set as a central zone. The minimum percentage of animal that must have been in the zone for an entry to occur was set at 70%, and 50% of the animal must have remained in the zone for an exit not to occur.

The effects of DMCM (2 mg/kg), 3-EBC (15 mg/kg) and β-CCt (15 mg/kg) on locomotor activity were evaluated, as well as the influence of β-CCt (15 mg/kg) on the effects of DMCM (2 mg/kg).

2.5. Rotarod performance

Rotarod test (Ugo Basile, Comerio, Italy) measured the capacity of the animal to maintain itself on the rod revolving 10 rpm. During two consecutive days, the rats were trained to walk on the revolving rod until they could complete three 180 s sessions (each day) without falling off. In the morning of the third day, the final selection was made. Only animals capable of

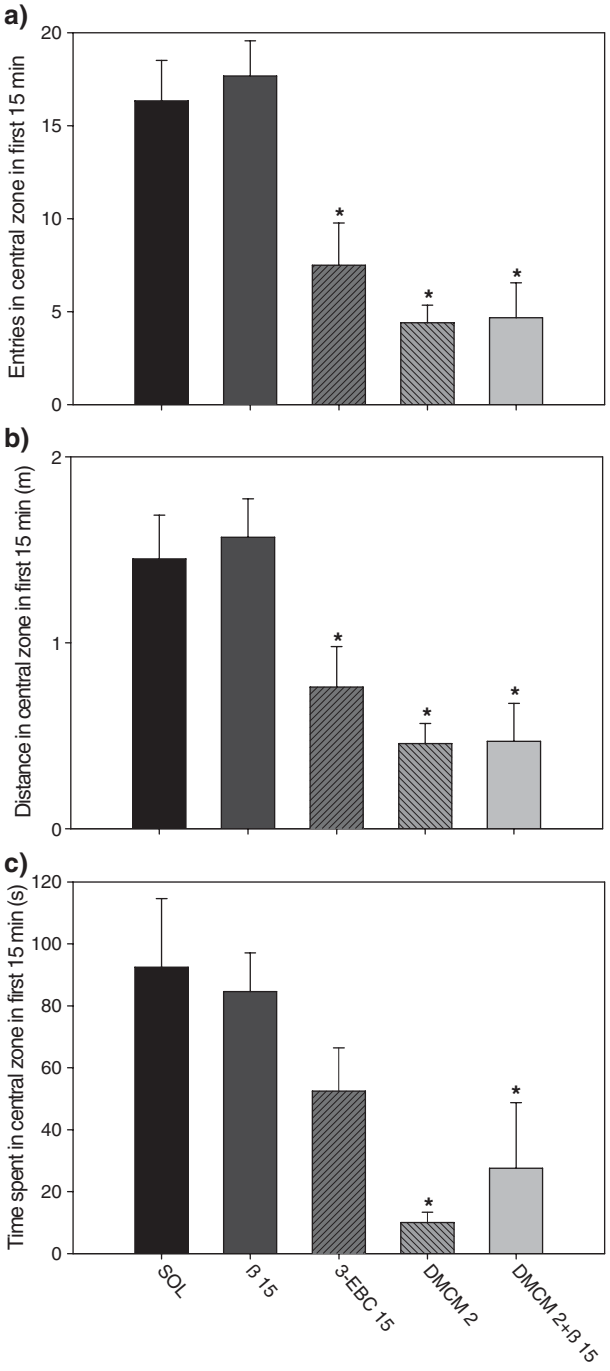

Fig. 5. The effects of β-CCt (β: 15 mg/kg), 3-EBC (15 mg/kg), DMCM (2 mg/kg), and combination of DMCM (2 mg/kg)+β-CCt (β: 15 mg/kg) on a) entries in central zone, b) distance travelled in central zone and c) time spent in central zone of the activity chamber during the first 15 min. *$p<0.05$ compared to solvent (SOL) group.

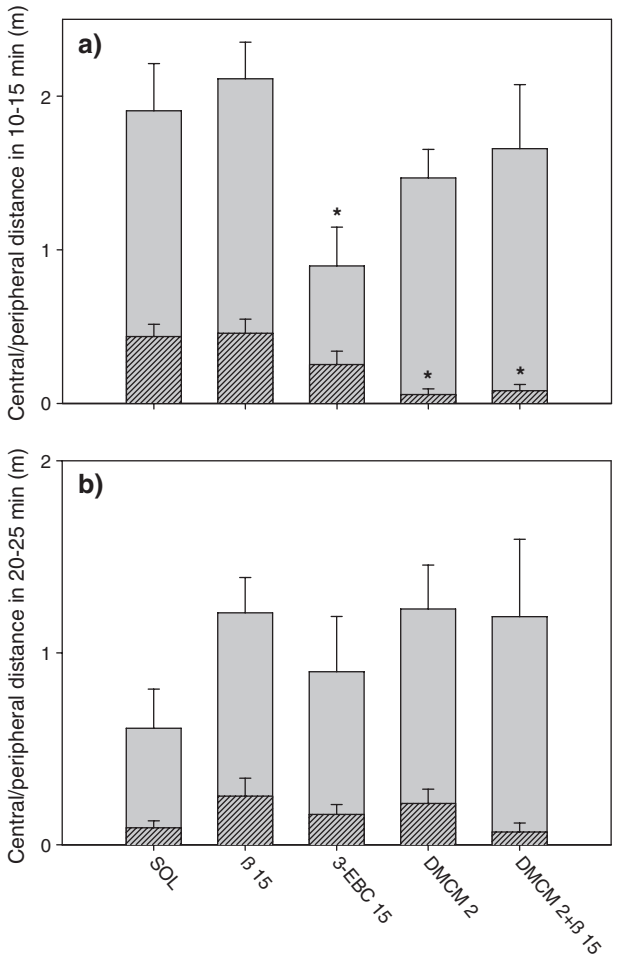

Fig. 6. The effects of β-CCt (β: 15 mg/kg), 3-EBC (15 mg/kg), DMCM (2 mg/kg), and combination of DMCM (2 mg/kg)+β-CCt (β: 15 mg/kg) on distance travelled in the central (hatched bars) and peripheral (open bars) zone of the activity chamber in the period a) 10–15 min and b) 20–25 min. *$p<0.05$ compared to solvent (SOL) group.

remaining 180 s on the rod, without any fall, were chosen for further testing. Those animals without capability to meet the same criterion 20 min after treatment were considered incapacitated.

The median incapacitating doses of DMCM, 3-EBC and β-CCt were planned to be determined.

2.6. Statistical analysis

All numerical data presented in the figures were given as the mean±SEM. Data from the EPM and activity assay were assessed by a one-way ANOVA. If the ANOVA was significant, each treatment condition was compared with control by a Dunnett's test ($\alpha=0.05$). Where appropriate, the assessment of the antagonist influence on the inverse agonist effect was conducted by a Student *t* test. The same test was used for comparisons in 5-min blocks from the activity assay. ED_{50} value for rotarod incapacitation was calculated by probit analysis according to the method of Litchfield and Wilcoxon. Statistical analyses were performed with ANY-maze Video Tracking System software (Stoelting Co., Wood Dale, IL, USA).

3. Results

3.1. Elevated plus maze

3.1.1. Activity-related parameters (Fig. 1)

Statistical analysis by ANOVA showed a significant effect of treatment on entries in closed arms ($F(3, 36)=6.80$, $p=0.001$). Dunnett's post hoc test revealed the significant effect of all three treatments in comparison to solvent group (Fig. 1a). The same stands for the parameter of total entries ($F(3, 36)=5.65$, $p=0.003$) (Fig. 1b) as well as for total distance travelled ($F(3, 36)=6.51$, $p=0.001$) (Fig. 1c).

There was no significant difference between activity levels induced by 3-EBC and 3-EBC+β-CCt; however, the effect of 3-EBC on entries in the closed arms was diminished by β-CCt (Fig. 1a).

3.1.2. Anxiety-related parameters

There was a significant effect of treatment on distance travelled in open arms ($F(3, 36)=3.91$, $p=0.016$), with the significant effect of DMCM related to control (Dunnett's test) (Fig. 2a). The influence of treatment on the percentage of open-arm entries did not reach statistical significance ($F(3, 36)=2.19$, $p=0.106$) (Fig. 2b), whereas the influence on the percentage of time on open arms was significant ($F(3, 36)=4.39$, $p=0.010$), with DMCM (2 mg/kg) being an effective treatment (Dunnett's test) (Fig. 2c).

As regards the combined treatment with 3-EBC and β-CCt, the addition of β-CCt significantly decreased the percentage of time spent in open arms, in comparison to 3-EBC on its own (Fig. 2c); also, there was a non-significant tendency to decrease the distance travelled and percentage of entries in open arms (*p* values 0.082 and 0.155, respectively).

3.2. Motor activity assay

3.2.1. Whole chamber-activity

An ANOVA showed a significant effect of treatment on total distance travelled during 45 min of monitoring ($F(4, 39)=3.16$, $p=0.024$) (Fig. 3). According to Dunnett's test, the activity-enhancing effect of β-CCt appeared to be significant related to solvent. When the analysis of travelled distance was developed into 5-min intervals (Fig. 4), it turned out that DMCM and 3-EBC decreased locomotion in periods 5–15 min and 10–15 min, respectively. On the other hand, DMCM increased locomotion in interval 20–25 min and β-CCt in intervals 5–10 and 15–25 min. The addition of β-CCt antagonized the hypolocomotor effect of DMCM in period 5–10 min post-treatment. Also, the plateau phase of DMCM-modulated activity present in period 10–30 min disappeared when β-CCt was co-administered.

3.2.2. Activity in the central zone

Statistical analysis by ANOVA revealed a clear tendency of treatment to decrease number of entries, distance travelled and time spent in the central zone during the whole 45-min period of recording; however, neither of the single treatments was

significant in this regard, according to Dunnett's test (data not shown). Furthermore, the analysis of central zone activity in the first 15 min was performed (Fig. 5). Namely, in a number of experiments it has been noticed that the inhibitory effects of β-carbolines on motor performance are present mainly during the first 15 min of recording (Jaskiw et al., 2003; Atack et al., 2005). Three parameters of central zone activity in the first 15 min (number of entries, distance travelled and time spent in central zone, Fig. 5a, b and c, respectively) were highly significantly ($p \leq 0.001$) decreased by treatment [$F(4, 39)$ values 11.19, 7.02 and 5.75, respectively]. According to Dunnett's test, DMCM on its own and in combination with β-CCt, as well as EBC, decreased parameters of central zone activity. However, the influence of 3-EBC on time spent in the central zone did not reach statistical significance (Fig. 5c).

3.2.3. Central/peripheral distance travelled in selected periods

Since inspection of Fig. 4 showed that there were two distinct periods: 10–15 min and 20–25 min after treatment, which may be seen to correspond to the peaks of hypo- and hyperlocomotor influences, respectively, the ratios of distance travelled in central and peripheral parts of the chamber in these periods are presented in the Fig. 6. For the period 10–15 min (Fig. 6a), there was a significant effect of treatment on distance travelled in the central zone ($F(4, 39)=6.21$, $p=0.001$), with the significant effects of DMCM and DMCM+β-CCt related to control (Dunnett's test). Also, the ANOVA showed a significant effect of treatment on distance travelled in the peripheral zone ($F(4, 39)=3.27$, $p=0.021$), with the significant effect of 3-EBC related to control (Dunnett's test). On the other hand, the analysis of central and peripheral distance travelled in the period 20–25 min after treatment (Fig. 6b) did not reveal any significant difference.

3.2.4. Rotarod test

When monitoring influence on rotarod performance as a quantal phenomenon (i.e. all or none effect) (Savić et al., 2003), the minimal incapacitating doses of DMCM and 3-EBC were 2 mg/kg and 45 mg/kg, respectively. At doses up to 90 mg/kg, β-CCt did not affect motor performance. The median incapacitating doses of DMCM and 3-EBC were 2.87 mg/kg and 52.07 mg/kg, respectively.

4. Discussion

The present experiments demonstrated that the non-selective inverse agonist DMCM and the preferential α_1-subunit affinity-selective inverse agonist 3-EBC differ in profiles of their influence on activity parameters in two unconditioned paradigms in rats.

In the EPM test, general activity-related parameters were decreased to a similar extent, whereas only DMCM affected open-arm parameters thought to reliably reflect anxiety. A previous testing of 3-EBC in the mouse EPM revealed a moderate potential of the anxiogenic influence of this β-carboline (Trullas et al., 1988). Moreover, a similar tendency was noticed in our pilot examination, performed without automated tracking. The lack of such a trend in the present study may be related with well-known variances in effects in the EPM test (cf. Dalvi and Rodgers, 1999). Nevertheless, the use of automated tracking and basal activity at an expected level reinforces the validity of the present findings. Addition of the α_1-preferring antagonist β-CCt to 3-EBC gave rise to a clear tendency to inhibit open-arm activity, without significant influence on general locomotor activity. Hence, it appears as if the potential of 3-EBC to induce an anxiogenic effect has been masked in some way by the predominant α_1-subunit-mediated actions of 3-EBC. On the basis of recent findings on DMCM-β-CCt interaction in the EPM (Savić et al., 2004), the relative significance of the α_1-subunit in the anxiogenic effects of DMCM could not have been clearly deduced, as the partial antagonism of the action of DMCM on anxiety-related parameters exerted by β-CCt was accompanied with a tendency of decreasing the activity-related parameters. The present data suggest that for the anxiogenic-like action of a benzodiazepine site inverse agonist in the EPM, subunits other than the α_1 need to be negatively modulated; a recent pharmacological study pointed to the α_3 subtype (Atack et al., 2005). On the other hand, inhibition of general activity-related parameters in the EPM by an inverse agonist (Cole et al., 1995; Atack et al., 2005) appears to involve both the α_1 and non-α_1 subtypes, with the probable exception of the α_5 subtype (Dawson et al., 2006). To this end, inhibition of general activity elicited by inverse agonists is clearly not a pure sedation, but may reflect a vague concept of drug-induced "lack of well being" (Atack et al., 2005) or decrease in exploratory drive.

In the locomotor assay, DMCM as well as 3-EBC induced depression of locomotion in the first recording period, up to 15 min after injection. Similarly, Atack et al. (2005) found the greatest inhibitory effects in a rat response sensitivity test (chain-pulling) 5–15 min after injection of two inverse agonists, α3IA and FG 7142. This effect of α3IA was seen at the dose which produced only 12% of occupation in the cerebellum, where the α_1-containing receptors predominate (Atack et al., 2005). On the contrary, it was shown that 3-EBC exhibits 1.5-fold and 2.5-fold greater affinity for rat cerebellum than for cerebral cortex and hippocampus, respectively (Trullas et al., 1988). The partial antagonism of the exploration-suppressing effect of DMCM by β-CCt adds evidence to the supposition that, as in the EPM, a decrease in immediate exploration of novelty induced by an inverse agonist should involve the α_1-subunit besides the $\alpha_{2/3}$ subtypes.

Following habituation to the test environment, greater activity levels were displayed in DMCM-treated rats than control rats. This difference reached significance in the period 20–25 min, and could not be dominantly ascribed to changes in activity in either of two virtual parts of the chamber. Crestani et al. (2002) reported that treatment with the inverse agonist Ro 15-4513 produced an increase in locomotor activity in a familiar context. When tested in α_1-knock in mice, the effect of the β-carboline was opposite — the compound displayed motor depressant effect, suggesting the role of the α_1-subunit in mediating the motor stimulant actions of that compound (Crestani et al., 2002). As the lagged plateau phase of DMCM-elicited activity disappeared by adding β-CCt, the

hypothesis on the role of α_1-containing $GABA_A$ receptors in hyperlocomotion induced by an inverse agonist appears applicable to the present findings as well. Although the lack of hyperlocomotion induced by the preferential α_1-subunit selective inverse agonist 3-EBC apparently does not conform to this hypothesis, it may be a consequence of clear differences in behavioral profiles of DMCM and 3-EBC in the habituation period; in other words, the proposed role of α_1-containing $GABA_A$ receptors in hyperlocomotion mostly applies to the non-selective inverse agonists.

The increase of distance travelled in the chamber elicited by β-CCt, in total and in the first 25 min, reproduced the hyperlocomotor effect of this β-carboline dosed at 30 mg/kg, observed during 10-min recording in an activity assay in mice, without habituation to the testing cage (DeLorey et al., 2001). On the other hand, when tested in rats, in similar settings, β-CCt dosed at 15 mg/kg was devoid of overt influences on locomotor activity (June et al., 2003). Although DeLorey et al. (2001) related locomotor-enhancing effect with inverse agonist activity, this explanation could hardly be probable. Namely, a depressant, rather than stimulant influence of an inverse agonist should be expected when the animal explores a novel testing area (Novas et al., 1988; Nagatani et al., 1990; Otter et al., 1997; Pahkla et al., 2000; Jaskiw et al., 2003). Accordingly, DeLorey et al. (2001) recorded a slight decrease of locomotion under DMCM (2 mg/kg). Otherwise, observing behavioral activity of benzodiazepine site antagonists in different tests is not at all unusual, and may be explained by the influence on the actual conformational equilibrium during testing, with or without implicating putative endogenous ligands (File and Pellow, 1986; Malizia and Nutt, 1995).

As regards the parameters of locomotion in the arbitrarily separated central zone of the activity chamber, there was a clear tendency to avoid this area when both inverse agonists as well as combination treatment were applied. However, the influence of 3-EBC was relatively less pronounced, and this treatment did not significantly decrease time spent in the central zone in first 15 min. The fact that antagonism of the α_1-subunit-mediated effects of DMCM by β-CCt did not affect the central-zone avoidance may mean that the non-α_1-subunits are involved in this effect. In line with this view, analysis of central/peripheral activity in the period 10–15 min revealed that DMCM and DMCM+β-CCt induced the reduction of distance travelled in the central zone, whereas 3-EBC reduced activity in the peripheral zone only. In the absence of data for receptor occupancy by 15 mg/kg of 3-EBC, it is not possible to unequivocally attribute the effect of this inverse agonist mainly to the α_1-subunit-containing receptors. However, the high minimal incapacitating dose of 3-EBC in the rotarod test (45 mg/kg) could be seen as indirect evidence for predominance of α_1-mediation of its effects at lower doses. Namely, non-selective inverse agonists, such as β-CCM, impaired rotarod performance at the dose (1 mg/kg) close to those affecting locomotion (Nagatani et al., 1990), anxiety level, cognition or epileptic activity (Chapouthier and Venault, 2003). For comparison, the median proconvulsive dose of 3-EBC (7 mg/kg) (Trullas et al., 1988) is 6.5 fold lower than the rotarod incapacitating one. As data from genetic studies suggest that impairment of motor performance by diazepam is mediated by non-α_1-subunits (Rudolph et al., 1999), the effects on general motor activity seen in this study may be mainly, but surely not exclusively, attributed to the α_1-mediated action of 3-EBC.

In conclusion, the present study confirms that benzodiazepine site inverse agonists may exert bimodal, hypo- as well as hyperlocomotor effect on activity parameters. Decreases of locomotor activity may presumably reflect an increase in emotional reactivity, i.e. anxiety, and be manifested in exposed parts of an apparatus (open arms in the EPM, central parts of an activity chamber), or a more vague "lack of well being" (Atack et al., 2005) or decrease in exploratory drive, and be manifested in more protective parts of the testing area (closed arms in the EPM, peripheral parts of a cage). Inhibition of activity in exposed areas may be mediated by non-α_1-subunits, whereas both α_1 and non-α_1-subunits may participate in behavioral suppression manifested in more protective areas. On the other side, the hyperlocomotor effect may be elicited in animals habituated to the testing area. This psychostimulant-alike effect might be dependent mainly on negative modulation at the α_1-containing receptors. Finally, the bimodal influence of inverse agonists on motor activity can be manifested as biphasic, i.e. observable in the same experiment: the initial decrease in parameters of locomotion may be followed by enhancement of locomotion in post-habitation period.

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