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Antidepressant-like effect of Ro5-4864, a peripheral-type benzodiazepine receptor ligand, in forced swimming test

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Abstract

This study examined the effects of peripheral-type benzodiazepine receptors in the forced swimming test. PK 11195 (1-(2-chloro-phenyl)-*N*-methyl-*N*-(1-methylpropyl)-1-isoquinoline carboxamide) and Ro5-4864 (7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepine-2-one) were i.p. injected in mice, according to an acute (1 or 24 h) and a repeated (14 days) schedule. Pretreatment with the agonist, Ro5-4864, significantly reduced immobility time 1 h after treatment but not 24 h after it, whereas the antagonist, PK11195, did not interfere with the test parameters. Nevertheless, PK11195 pretreatment inhibited the Ro5-4864 antidepressant-like effect. Animals repeatedly treated with Ro5-4864 had a similar profile of action with no sign of motor impairment or locomotor activation as evaluated in the rota-rod and open-field tests, respectively. Aminoglutethimide pretreatment, which blocks the early step of steroid synthesis, inhibited the antidepressant-like effect of Ro5-4864. The present findings suggest an antidepressant-like profile for the benzodiazepine, Ro5-4864, that seems to involve steroid synthesis as underlying mechanism.

Keywords: Benzodiazepine receptor, peripheral-type; PK11195; Ro5-4864; Depression; Steroid; (Mouse); Forced swimming test

1. Introduction

The benzodiazepines are among the most prescribed drugs because of their anxiolytic, anticonvulsant and sedative actions. On the basis of the selectivity for their ligands and subcellular localisation, benzodiazepine-binding sites can be divided into central and peripheral types. Central-type benzodiazepine receptors occur only in neurons in the central nervous system whereas peripheral-type benzodiazepine receptors are detected mainly in the outer mitochondrial membranes in various tissues including the central nervous system (Anholt et al., 1986; Veenman and Gavish, 2000; Casellas et al., 2002). The therapeutic activities (anxiolytic, hypnosedative, anticonvulsant and muscle relaxant effects) of benzodiazepines are attributed to an action on central-type benzodiazepine receptor sites. In fact, diazepam binds with nearly similar affinity to both receptors, but other ligands, such as the benzodiazepine, Ro5-4864, and the isoquinoline, PK11195, show selectivity. These last compounds have high affinity for the peripheral-type benzodiazepine receptors but do not bind to the central-type benzodiazepine receptors site (Le Fur et al., 1983; Schoemaker et al., 1983). High levels of peripheral-type benzodiazepine receptors have been found in several peripheral tissues such as steroidogenic organs and blood cells (Papadopoulos et al., 1990). Peripheral-type benzodiazepine receptor ligands stimulate steroid synthesis in adrenal (Mukhin et al., 1989), placental (Barnea et al., 1989), testicular, ovarian tissues (Amsterdam and Suh, 1991) and glial cells (Papadopoulos et al., 1990) by translocation of cholesterol from outer to inner mitochondrial membranes, the rate-limiting step in steroidogenesis (Krüeger, 1991).

In the brain, peripheral-type benzodiazepine receptors are predominantly localised on glial cells (Costa and Guidotti, 1991). Neurosteroids, so-called because they are produced in the nervous system, are synthesised particu-

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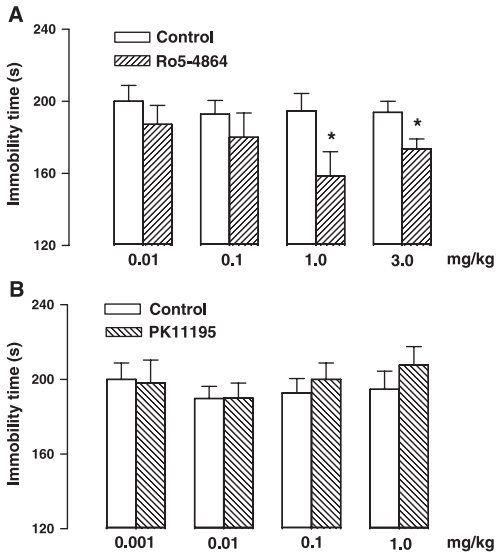

Fig. 1. Effect of the pretreatment 1 h before the test with the benzodiazepine, Ro5-4864 (0.01 to 3.0 mg/kg—A) and the isoquinoline, PK11195 (0.001 to 1.0 mg/kg—B) on the immobility time in the forced swimming test, recorded for 5 min in mice. Each value represents the mean ± S.E.M. for 7–10 animals. *$P<0.05$ as compared to control group (one-way ANOVA followed by Newman–Keuls' test).

larly in glial cells. Evidence suggests that neurosteroids could play a role in a variety of neural and affective functions and disorders, such as memory, stress, anxiety, depression and others (Majewska, 1992). Since peripheral-type benzodiazepine receptor ligands activate neurosteroids synthesis, this evidence suggests a putative role of these molecules in the treatment of functional central nervous system disorders.

Our preliminary studies examined the participation of peripheral-type benzodiazepine receptors in experimental models of central nervous system disorders. In the elevated plus-maze test, which models anxiety-like behaviours (Lister, 1990), i.c.v. administration of peripheral-type benzodiazepine receptors ligands showed an anxiogenic-like effect. Both ligands were also effective to reverse electroshock-induced seizures, showing an anticonvulsant effect. Furthermore, Ro5-4864 reduced immobility time in the forced swimming test, suggesting an antidepressant-like profile for this benzodiazepine (Gavioli et al., personal communication). Thus, the present study aimed to further verify the participation of peripheral-type benzodiazepine receptors in the modulation of an experimental depression model in mice using PK11195 and Ro5-4864 as peripheral-type benzodiazepine receptor ligands. In order to investigate the participation of adrenal glucocorticoids in the behavioural effect of these compounds, animals were pretreated with aminoglutethimide, an enzyme inhibitor which blocks the early step of steroidogenesis (Mazzocchi et al., 2002). To reach our goals, we used the forced swimming test, a well-established setup for screening potential antidepressant agents (Porsolt et al., 1977a,b).

2. Material and methods

2.1. Animals

Adult Swiss male mice (25–30 g) were housed in groups of 20 animals per cage and kept on a controlled 12-h light/dark cycle (lights on at 07:00 AM) and temperature (22 ± 1 °C). The animals had free access to standard pellet food and water, except during the experiments. At least 24 h before testing, mice were held in the laboratory where the experiments were carried out. Each animal was drug-naive and was used only once. Each drug was administered to 8–10 mice. Experiments were performed between 08:00 AM and 01:00 PM. All experiments were conducted in accordance with international standards of animal welfare recommended by the Brazilian Society of Neuroscience and Behaviour (Act, 1992) and approved by the University Committee for Animal Care in Research (#23080.001156/2001-50/UFSC). The minimum number of animals and duration of observation required to obtain consistent data were used.

2.2. Drugs

Peripheral-type benzodiazepine receptor ligands: PK 11195 (1-(2-chloro-phenyl)-*N*-methyl-*N*-(1-methylpropyl)-1-isoquinoline carboxamide) or Ro5-4864 (7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one) were kindly donated by Sanofi Recherche. They were dissolved in absolute ethanol, and freshly diluted in saline containing 0.5% Tween 80 to the adequate concentrations at the beginning of treatments. Imipramine, the standard antidepressant compound, and aminoglutethimide, the cholesterol side-chain-cleaving enzyme inhibitor, were purchased from Sigma (MO, USA) and were dissolved in saline and DMSO (dimethylsulphoxide) 10%, respectively.

2.3. Drug administration

The animals received i.p. injections of peripheral-type benzodiazepine receptors ligands, PK11195 and/or Ro5-

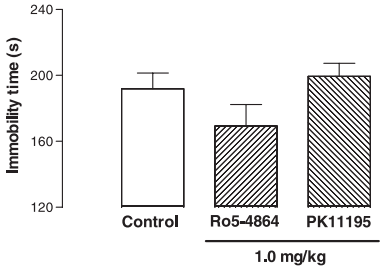

Fig. 2. Effect of the pretreatment 24 h before the test with the benzodiazepine, Ro5-4864 (1.0 mg/kg), and the isoquinoline, PK11195 (1.0 mg/kg), on the immobility time in the forced swimming test, recorded for 5 min in mice. Each value represents the mean ± S.E.M. for 8–10 animals. $P>0.05$ as compared to control group (one-way ANOVA).

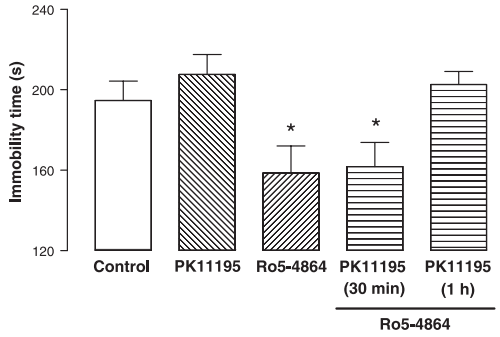

Fig. 3. Effect of the pretreatment with the isoquinoline, PK11195 (1.0 mg/kg), 30 min and 1 h before the treatment with the benzodiazepine, Ro5-4864 (1.0 mg/kg), on the immobility time in the forced swimming test, recorded for 5 min in mice. Each value represents the mean ± S.E.M. for 8–10 animals. *$P<0.05$ as compared to control group (one-way ANOVA followed by Newman–Keuls' test).

4864, or saline (control group) with adequate concentrations of ethanol (control group). One hour (PK11195: 0.001 to 1.0 mg/kg; Ro5-4864: 0.01 to 3.0 mg/kg) or 24 h (1.0 mg/kg of both compounds) after the acute treatment, the effects were evaluated in the forced swimming test. Imipramine (15 mg/kg) was used as standard antidepressant drug and was i.p. injected 30 min before the test. Aminoglutethimide (10 mg/kg) was i.p. injected 30 min before the Ro5-4864 treatment. In the repeated treatment experiment, the animals were treated for 14 days with Ro5-4864 (1.0 mg/kg) and tested in the forced swimming test 1 h after the last drug administration. Imipramine (15 mg/kg), the standard antidepressant drug, was used according to the same repeated schedule.

2.4. Forced swimming test

To measure immobility, we essentially followed the method proposed by Porsolt et al. (1977a). Briefly, animals had a swimming-stress session for 15 min (pre-test), 24 h before being individually placed in plastic cylinders (height

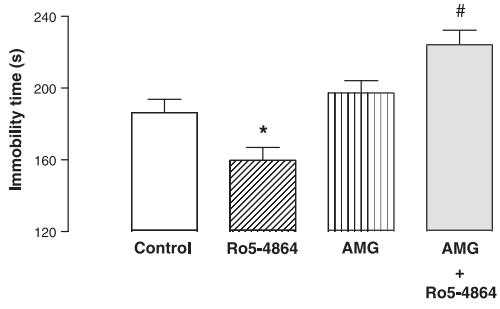

Fig. 4. Effect of the pretreatment with the inhibitor of steroid synthesis, aminoglutethimide (AMG; 10 mg/kg), 30 min before the treatment with the benzodiazepine, Ro5-4864 (1.0 mg/kg), on the immobility time in the forced swimming test, recorded for 5 min in mice. Each value represents the mean ± S.E.M. for 8–10 animals. *$P<0.05$ as compared to control group and # as compared to Ro5-4864 group (one-way ANOVA followed by Newman–Keuls' test).

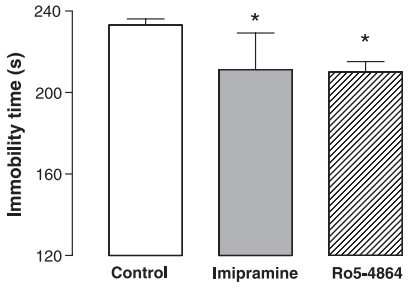

Fig. 5. Effects of repeated treatment (14 days) with the benzodiazepine, Ro5-4864 (1.0 mg/kg), and imipramine (15 mg/kg) on the immobility time in the forced swimming test, recorded for 5 min in mice. Each value represents the mean ± S.E.M. for 7–10 animals. *$P<0.05$ as compared to control group (one-way ANOVA followed by Newman–Keuls' test).

18.5 cm, diameter 12.5 cm containing 17 cm of water at 23 ± 2 °C) for 5 min (test). In the test session, 1 or 24 h after the acute treatment or 1 h after the end of the repeated treatment, behaviour was observed for up 5 min by an experienced observer, who was blind to the treatment conditions. Animals were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their head above water.

2.5. Open field test

To evaluate the influence of the treatment i.p. with peripheral-type benzodiazepine receptor ligands on spontaneous locomotor activity, the mice were placed individually, 1 h after the injection, in the centre of a Plexiglas arena (35 × 35 × 15 cm) for 5 min and the numbers of crossings and rearings were recorded. In the repeated treatment experiment, locomotor activity was assessed immediately before the swimming test.

2.6. Rota-rod test

Mice were placed on the horizontal rotating bar (diameter 2.5 cm, 12 rpm) of the rota-rod apparatus 1 or 24 h after the treatments. The total time spent on the bar during a 1-min session was recorded using a stopwatch, and the number of

Table 1
Effect of the acute pretreatment with the benzodiazepine, Ro5-4864 or PK11195 (1.0 mg/kg), 1 h before the test on the parameters evaluated in the open field and rota-rod tests, recorded for 5 and 1 min, respectively, in mice

Treatment	Locomotor activity		Rota-rod	
	Crossings	Rearing	Number of falls	Time on the rotation bar (s)
Control	76.9 ± 5.2	33.7 ± 3.1	0.4 ± 0.3	57.0 ± 1.9
Ro5-4864 1.0 mg/kg	82.8 ± 10.7	30.0 ± 2.7	0.3 ± 0.2	56.7 ± 2.2
PK11195 1.0 mg/kg	73.7 ± 9.89	24.7 ± 2.7	1.0 ± 0.5	54.7 ± 3.1

Each value represents the mean ± S.E.M. for 8–10 animals ($P>0.05$; one-way ANOVA).

falls during the session was also recorded (Dunham and Miya, 1957). In the repeated treatment experiment, the rota-rod test was run immediately after the open-field test.

2.7. Data analysis

Data were expressed as means ± S.E.M. The means were compared using the one-way analysis of variance ANOVA (independent variable = drug treatment) followed by Newman–Keuls' test. Under our experimental conditions, the differences were considered to be significant when P was < 0.05.

3. Results

As shown in Fig. 1A, Ro5-4864 (0.01 to 3.0 mg/kg i.p.) significantly reduced immobility time at the highest doses (1.0 and 3.0 mg/kg), 1 h but not 24 h after the pre-treatment (Fig. 2). These findings indicated an antidepressant profile of action for this compound. The reference antidepressant drug, imipramine (15 mg/kg), also reduced immobility time in the forced swimming test run 30 min after the i.p. treatment (control: 193 ± 9 s; imipramine: 138 ± 13 s).

PK11195 (0.001 to 1.0 mg/kg), on the other hand, did not alter the immobility time at 1 or at 24 h after the i.p. treatment at any dose used (Figs. 1B and 2). Moreover, pretreatment with PK11195 i.p. 1 h, but not 30 min before, inhibited the antidepressant-like effect of Ro5-4864 as depicted in Fig. 3, indicating that the benzodiazepine, Ro5-4864, has an agonist profile of action after acute treatment, whereas the isoquinoline, PK-11195, has an antagonist profile of action in the forced swimming test in mice.

Pretreatment with aminoglutethimide, a cholesterol side-chain-cleaving enzyme which blocks the early step of steroid synthesis, has no behavioural effect per se but blocked the antidepressant-like activity of Ro5-4864, as depicted in Fig. 4.

As shown in Fig. 5, repeated treatment with Ro-5-4864 (1.0 mg/kg) for 14 days reduced the immobility time of mice, when compared to control values, as was seen after its acute administration as well as after the repeated treatment with imipramine.

As shown in Tables 1 and 2, the acute and repeated treatment with Ro5-4864 or imipramine and the acute treatment with PK11195 (1.0 mg/kg) did not modify locomotor activity or motor coordination of mice evaluated in open field and rota-rod tests, respectively.

Table 2
Effects of repeated treatment (14 days) with the benzodiazepine, Ro5-4864 (1.0 mg/kg), and imipramine (15.0 mg/kg) on locomotor activity and motor performance evaluated in the open field and rota-rod tests, respectively, in mice

Treatment	Locomotor activity		Rota-rod	
	Crossings	Rearing	Number of falls	Time on the rotation bar (s)
Control	78.7 ± 5.7	42.1 ± 3.2	1.2 ± 0.3	56.4 ± 1.0
Ro5-4864 1.0 mg/kg	78.6 ± 7.6	42.7 ± 2.9	0.9 ± 0.3	57.3 ± 1.0
Imipramine 15.0 mg/kg	80.1 ± 8.3	36.4 ± 2.6	0.8 ± 0.4	56.7 ± 1.5

Each value represents the mean ± S.E.M. for 8–10 animals (P>0.05; one-way ANOVA).

4. Discussion

To our knowledge, this is the first demonstration that the benzodiazepine, Ro5-4864, but not the isoquinoline, PK11195, agonist and antagonist at the peripheral-type benzodiazepine receptors, respectively, has an antidepressant-like profile of action after acute and repeated treatment in the classical Porsolt's model of forced swimming. Porsolt et al. (1977a,b, 1978), who proposed this behavioural model to screen new antidepressant compounds, concluded that the immobility observed reflected a state of lowered mood or hopelessness in animals. However, there is some evidence for a lack of specificity of the test because several drugs could reduce the duration of immobility in mice (Browne, 1979; Schechter and Chance, 1979; Wallach and Hedley, 1979). Kitada et al. (1981) showed, though, that acute and chronic administration of antidepressants reduced the duration of immobility by prolonging the escape-directed behaviour during the first 5 min of the forced swimming test and that potentiation of the effect after chronic treatment is an action specific to antidepressants. A recent review of animal models for assessing antidepressant activity (Cryan et al., 2002) discussed the use of the forced swimming test and concluded that, for several reasons, this is still the most widely used tool for pre-clinical screening, although it does not reliably detect 5-hydroxytryptamine re-uptake inhibitors.

The antidepressant-like effect of Ro5-4864 was observed 1 h but not 24 h after the acute i.p. treatment which is consistent with the literature data showing that acute stress promotes a marked reduction of the density of peripheral-type benzodiazepine receptors after 1 h (Avital et al., 2001). Moreover, repeated treatment confirmed the antidepressant-like profile of action of Ro5-4864. In this regard, Leschiner et al. (2000) showed that the 21-day treatment with antidepressants induced a significant decrease in peripheral-type benzodiazepine receptor density in the adrenals, kidney, liver and cerebral cortex, although the relevance of these changes for the antidepressant agents remains to be clarified. In spite of it, no changes of peripheral-type benzodiazepine receptor number were observed in patients with major depression (Weizman et al., 1995), obsessive–compulsive disorder (Marizziti et al., 1994) or schizophrenia (Weizman et al., 1986). However, experimental results suggest that these receptors play a role in physiological adaptation to stress, anxiety and depression (for review, see Gavish et al., 1999). In fact, a long-term period of tail shock produced a decrease of peripheral-type benzodiazepine receptors in the cerebral

cortex (Novas et al., 1987) and rats used for the acute swim stress showed a rapid increase in peripheral-type benzodiazepine receptor levels in the olfactory bulb (Drugan et al., 1986; Basile et al., 1987). Thus, the forced swimming test could be considered a stressor and the effect observed here could be a consequence of an anxiolytic-like action of Ro5-4864. Our preliminary studies (Gavioli et al., personal communication) had shown an anxiogenic-like profile for this compound as previously described in the literature (Holmes and Drugan, 1991; Mizoule et al., 1995). Moreover, tonic immobility, as characterised by a temporary state of profound and reversible motor inhibition, is sometimes used as an anxiety model since it is an innate fear response. In fact, tonic immobility used as an anxiety model is a terminal defence response occurring when there is physical contact between prey and predator, which is not the case in the forced swimming test (Olsen et al., 2002).

So far there are no reports of antidepressant activity of the peripheral-type benzodiazepine receptor ligands, except from the studies of Raghavendra et al. (2000). They showed that acute administration of Ro5-4864 (2.5 mg/kg i.p.), an agonist at these receptors, but not the antagonist, PK-11195 (1 mg/kg, i.p.) had an antidepressant-like profile in the chronic forced swim-induced immobility test. This profile of action was similar to that of melatonin and γ-aminobutiric acid receptor ligands also studied by these authors. PK11195, on the other hand, inhibited the antidepressant action of melatonin and Ro5-4864. Chronic or repeated forced swimming stress, as used by these authors, yields marked differences in the sensitivity of various behavioural and physiological responses when compared to the single test, as we have done (Dal-Zotto et al., 2000). These differences are interpreted as sensitisation of the hypothalamic–pituitary–adrenal axis and could interfere with the effects of Ro5-4864, whereas our results probably reflect a direct action of this compound on the peripheral-type benzodiazepine receptor sites, since our preliminary study showed the same profile of action after i.c.v. injection of Ro5-4864 and PK-11195 in the single forced swimming test (Gavioli et al., personal communication). Ro-5-4864 also presented a clear antidepressant-like profile of action after repeated treatment, as shown by the significant reduction of immobility time in the forced swimming test, confirming the acute effect. At the moment, it is not possible to state whether or not this effect is due to an interaction at the central nervous system peripheral-type benzodiazepine receptors, which remains to be further investigated.

Another possibility to explain the antidepressant-like effect reported here is the action of Ro5-4864 on the central-type benzodiazepine receptors. This hypothesis for the underlying mechanism of action is also under investigation, although we do not believe it is applicable since neither chlordiazepoxide nor diazepam (benzodiazepine compounds that bind to central-type receptors) affected the duration of immobility even at doses which produced marked ataxia (Porsolt et al., 1977a,b, 1978).

Many drugs are reported to affect any or several aspects of motor function, resulting in false assumptions about the drugs' effects. One of the simpler procedures widely used in the context of motor function, endurance and balance, is the rota-rod apparatus. The rota-rod provides reliable information about motor coordination and central/peripheral neurotoxicity in rodents. For this reason, in the present study animals were also evaluated in the open-field and rota-rod tests of investigate possible alterations in motor activity and/or performance, respectively. Neither Ro5-4864 nor PK-11195 alters the animal's performance in the rota-rod test, which makes it unlikely that the antidepressant-effect (or its lack) is consequence of any motor effects of peripheral-type benzodiazepine receptor ligands. The results obtained in the open-field test, where no change was observed in treated animals, also support the idea of a specific antidepressant-like effect of Ro5-4864.

On the other hand, the peripheral-type benzodiazepine (or mitochondrial DBI) receptors play a central role in the regulation of steroidogenesis (Anholt et al., 1986; Benavides et al., 1983). Brain neurosteroid levels seem to be linked to environmental and behavioural features, such as stress, aggressiveness and neuropsychiatric disorders (Majewska, 1992). It has been also shown that peripheral-type benzodiazepine receptors play a critical role in the production of neurosteroids (Krüeger and Papadopoulos, 1990) and, like PK11195 and Ro5-4864, stimulate pregnenolone synthesis in brain mitochondria (Krüeger and Papadopolous, 1992). Thus, as aminoglutethimide pretreatment, which blocks steroidogenesis at an early step, inhibited Ro5-4864's antidepressant-like effects, it could be hypothesised that behavioural effects of Ro5-4864 described here as well as its involvement in neuropsychiatric disorders could involve stimulation of steroid synthesis. This hypothesis led to the suggestion of a potential role of peripheral-type benzodiazepine receptor ligands in the treatment of functional central nervous system disorders. Furthermore, there is some evidence that neurosteroids also play a pivotal role in depression (Reddy et al., 1998).

Our results indicate a potential therapeutic use of peripheral-type benzodiazepine receptor ligands, particularly the benzodiazepine, Ro5-4864, an agonist at these sites, in the treatment of central nervous system disorders such as endogenous depression, since on acute treatment of mice it produced an antidepressant-like effect. Moreover, the peripheral-type benzodiazepine receptor antagonist, PK-11195, inhibited this action. The present findings also showed a putative participation of peripheral-type benzodiazepine receptors in steroidogenesis as the underlying mechanism of this action.

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