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European Journal of Pharmacology 482 (2003) 197–203
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Anxiolytic-like effects of the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 in non-human primates

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Received 1 July 2003; received in revised form 8 September 2003; accepted 30 September 2003

Abstract

Non-human primates provide important insights into the potential use of 5-HT$_{1A}$ receptor antagonists in treating human anxiety disorders and as research tools, given the existent inconsistencies in rodent tests. This study investigated the effects of the selective silent 5-HT$_{1A}$ receptor antagonist *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane-carboxamide trihydrochloride (WAY 100635), administered systemically, in an ethologically based fear/anxiety test in marmoset monkeys (*Callithrix penicillata*). Subjects were tested using a figure-eight maze and a taxidermized wild cat as 'predator' stimulus. After seven 30-min maze habituations in the absence of the 'predator', each animal was submitted to four pseudo-randomly assigned 30-min treatment trials in the presence of the 'predator': three WAY 100635 (0.2, 0.4 and 0.8 mg/kg, i.p.) sessions and a saline control trial. The 'predator' stimulus caused a significant fear-induced avoidance of the maze sections closest to where it was presented, indicating an anxiogenic effect. However, WAY 100635 treatment reversed, significantly and dose-dependently, this fear-induced avoidance behavior, while increasing maze exploration. Sedation was not observed. This is the first study to suggest an anxiolytic-like effect of the selective silent 5-HT$_{1A}$ receptor antagonist WAY 100635 in non-human primates, indicating its potential use as a therapeutic agent.

Keywords: WAY 100635; Marmoset, monkey; Figure-eight maze; Taxidermized predator

1. Introduction

The 5-HT$_{1A}$ receptor has been extensively investigated regarding its role in fear and anxiety (De Vry, 1995). In general, 5-HT ligands that stimulate postsynaptic 5-HT$_{1A}$ receptors in terminal areas of serotonergic projections have an anxiogenic profile (e.g., File et al., 1996). Compounds that stimulate inhibitory somatodendritic 5-HT$_{1A}$ autoreceptors in the raphe nuclei, on the other hand, decrease the firing frequency of 5-HT neurons and hence reduce 5-HT release, inducing anxiolytic effects (e.g., File et al., 1996).

Numerous investigations employing 5-HT$_{1A}$ receptor agonists (e.g., 8-hydroxy-2-(di-*N*-propylamino)tetralin(8-OH-DPAT), however, yielded highly variable results in different anxiety tests, particularly for systemically administered compounds (for a review, see Griebel, 1995). Controversial effects have also been reported for several 5-HT$_{1A}$ receptor antagonists in rodent tests of anxiety, as for instance 1-(2-methoxyphenyl)-4-(4-(2-phthalimido)butyl)-piperazine (NAN-190), 5-fluoro-8-hydroxy-2-(dipropylamino)tetralin ((*S*)-UH-301) and *N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperzin-1-yl)-2-phenylpropanamide (WAY 100135) (Moreau et al., 1992; Charrier et al., 1994; Rodgers and Cole, 1994; Griebel et al., 1999). Various 5-HT$_{1A}$ receptor antagonists were later shown to be non-selective and/or possess mixed agonist/antagonist activity (Arborelius et al., 1993; Assie and Koek, 1996; Routledge, 1996), which may partially account for some of these inconsistencies. Thus, the development of selective and silent 5-HT$_{1A}$ receptor antagonists, such as *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane-carboxa-

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doi:10.1016/j.ejphar.2003.09.064

mide trihydrochloride (WAY 100635) (Forster et al., 1995; Fletcher et al., 1996), proved essential for more conclusive investigations. In fact, WAY 100635 has been found to decrease terminal 5-HT concentrations after systemic administrations in rats (Hjorth et al., 1997; Müller et al., 2002a), suggestive of an anxiolytic potential for this compound. Surprisingly, investigations on the anxioselective profile of systemically administered WAY 100635 in different rodent tests of anxiety ranged from anxiolysis (Fletcher et al., 1996; Cao and Rodgers, 1997, 1998; Griebel et al., 1999, 2000) and no effect (Stanhope and Dourish, 1996; Bell et al., 1999), to anxiogenesis (Groenink et al., 1995).

Given the controversial findings in rodent tests of anxiety, studies with non-human primates may provide important insights into the potential use of selective 5-HT_{1A} receptor antagonists as therapeutic agents for human affective disorders (King et al., 1988). To our knowledge, the only previous primate study investigating the effects of a 5-HT_{1A} receptor antagonist reported in squirrel monkeys an anxiolytic-like action for (*S*)-UH-301, a non-specific 5-HT_{1A} receptor antagonist (Moreau et al., 1992). The aim of the present study, therefore, was to investigate the effects of the selective silent 5-HT_{1A} receptor antagonist WAY 100635 in an ethologically based fear/anxiety test in non-human primates. Based on previous neurochemical findings showing a 5-HT decrease in terminal areas after WAY 100635 treatment (Hjorth et al., 1997; Müller et al., 2002a), and the overall results of behavioral studies in different rodent tests of anxiety (e.g., Cao and Rodgers, 1997; Griebel et al., 2000), an anxiolytic effect was expected for this drug in non-human primates.

2. Materials and methods

2.1. Subjects

Five experimentally naive adult marmosets (*Callithrix penicillata*, two males and three females) were used as subjects. Animals weighed 300–400 g at the beginning of experiments, and all were socially housed in three separate male/female groups in indoor/outdoor cages (2 × 1.3 × 2 m) of the same colony room. In one group, only the female was used in this study. Maintenance and testing of subjects were performed at the Primate Center, University of Brasilia. Except during the brief 30-min test periods, food and water were available ad libitum. All procedures were approved by the Animal Ethics Committee of the Institute of Biology, University of Brasilia, Brazil, and followed the 'Principles of Laboratory Animal Care' (NIH Publication No. 85-23, revised 1996).

2.2. Drugs

WAY 100635 (Sigma, USA) was dissolved in 0.9% physiological saline and injected i.p. in the doses of 0.2, 0.4 and 0.8 mg/kg. The injection volume for WAY 100635 and saline injections (vehicle control) was 1 ml/kg. All treatments were administered in the animals' home cages. Dose range was based on previous behavioral experiments investigating the effects of WAY 100635 in rodent tests of anxiety (Cao and Rodgers, 1997, 1998; Griebel et al., 1999, 2000).

2.3. Apparatus

Testing was conducted in a figure-eight continuous maze, recently validated as an ethologically based apparatus to measure fear/anxiety in marmosets (for a review, see Barros and Tomaz, 2002). The maze consisted of a rectangular field (125 × 103 × 35 cm) suspended 1 m from the floor and divided into five arms by two holes and barriers, forming a continuous figure-eight maze (Fig. 1). The apparatus, made of 4 mm transparent glass on a metal frame support, was divided into two segments (front and back chambers) by a concrete visual barrier (147 × 8 × 218 cm). The back chamber consisted of an arm (125 × 30 × 35 cm) with a central guillotine-type door and removable barriers. The latter formed the start compartment. The front chamber had three parallel arms (40 × 25 × 35 cm), 25 cm apart, ending in a common perpendicular arm (125 × 25 × 35 cm). Both chambers were interconnected through holes in the visual barrier at each of the three parallel arms. A taxidermized wild oncilla cat (*Felis tigrina*), which is a potential natural predator of marmosets, was placed outside the maze facing one corner of the parallel arms. The concrete barrier prevented subjects from viewing the taxidermized cat as they entered the maze, enabling a casual encounter via

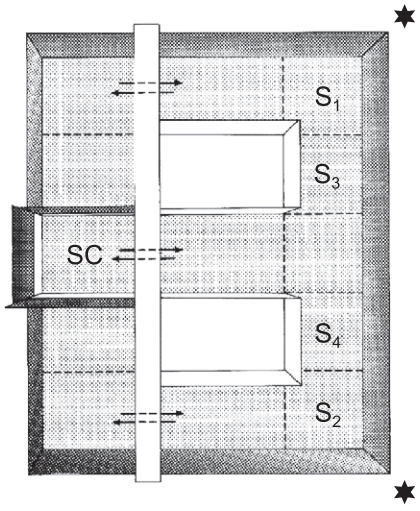

Fig. 1. Topview of the figure-eight maze used in the Marmoset Predator Confrontation Test of fear/anxiety. (SC) indicates the start compartment, the stars show the two locations where the taxidermized predator could be positioned, the dotted lines indicate the divisions of the maze into 13 sections, S_1 and S_2 correspond to the maze sections closest to the 'predator' location, and S_3 and S_4 are the maze sections immediately adjacent to the 'predator' position.

spontaneous exploration of the maze by the subject (for details, see Barros and Tomaz, 2002).

2.4. Procedure

2.4.1. Habituation trials

To avoid confounding effects of exposing the marmosets to a novel environment (i.e., maze) while measuring their response to a taxidermized predator, all subjects were first submitted to seven 30-min habituation trials, 48 h apart and in the absence of the 'predator'. These trials are essential to reliably measure the marmosets' fear/anxiety behavior in response to the 'predator' stimulus, as they predominantly display a highly erratic locomotor activity when first exposed to novel environments. This behavior declines to a baseline level prior to the seventh trial (Barros et al., 2000, 2001, 2002a). The procedure employed for the habituation trials consisted of the same protocol described below for subsequent trials, however, animals were not submitted to any treatment. Instead, subjects were only handled for 1 min and then placed in a transport cage (35 × 20 × 23 cm).

2.4.2. Treatment trials

Following the habituation trials, four pseudo-randomly assigned treatment trials were performed with each subject: three i.p. injections of WAY 100635 (0.2, 0.4 and 0.8 mg/kg) and a saline control. For each trial, the subject was quickly captured in its home cage, administered a treatment and placed thereafter into the transport cage. Following a 10-min interval, the marmoset was released into the maze's back chamber start compartment, thus commencing a 30-min trial. Barriers from this compartment were promptly removed upon the animal's exit, permitting free access to the whole apparatus. After the session, the subject was returned to its home environment in the transport cage. Overall, each marmoset received four i.p. injections (i.e., saline, 0.2, 0.4 and 0.8 mg/kg WAY 100635) spaced 72 h apart. During treatment trials, the 'predator' was present on either the left or right corner of the maze's back chamber (Fig. 1), and its position pseudo-randomly assigned to each subject, remaining constant throughout these trials. Treatments and order of subjects were pseudo-randomly assigned for each test day. Video cameras were used for online monitoring and all trials were recorded for later behavioral analysis. All test sessions were performed between 07:30 and 10:00 a.m.

2.5. Behavioral analysis

For behavioral analysis, the maze was divided into 13 sections (Fig. 1). The following behavioral parameters were scored for each 30-min trial by an experienced observer blind to the experimental treatment (intra-rater reliability ≥ 95%): (1) *exploratory activity*, the frequency of sniffing and/or licking any part of the apparatus, and/or leg stand (to raise the body into a bipedal position); (2) *proximity to 'predator'*, the frequency and time spent in the maze sections closest to (S_1 and S_2) and immediately adjacent to (S_3 and S_4) the 'predator' location (only the adjacent sections equal in size to S_1 and S_2 were analyzed; Fig. 1); and (3) *locomotor activity*, the number of maze sections crossed with both forelimbs. Locomotor activity and proximity to 'predator' were scored using a semi-automated behavior analysis program (Chromotrack 4.02, San Diego Instruments), whereas the frequency of exploratory activities was measured by focal-all occurrences samplings. Exploratory activity and proximity to 'predator' have been consistently shown as fear/anxiety measures in marmosets (e.g., Carey et al., 1992; Barros et al., 2002b), influenced by diazepam, buspirone and substance P in the same fear/anxiety test presently employed (Barros et al., 2000, 2001, 2002a). Locomotor activity was used as a measure of habituation to the maze, as well as to detect possible sedating or activating effects of WAY 100635.

2.6. Statistical analysis

Non-normally distributed data were log transformed. Exploratory and locomotor activity were analyzed by means of one-way analysis of variance (ANOVA) with repeated measures on the time (habituation trials) or treatment factor (treatment trials). Frequency and duration of proximity to

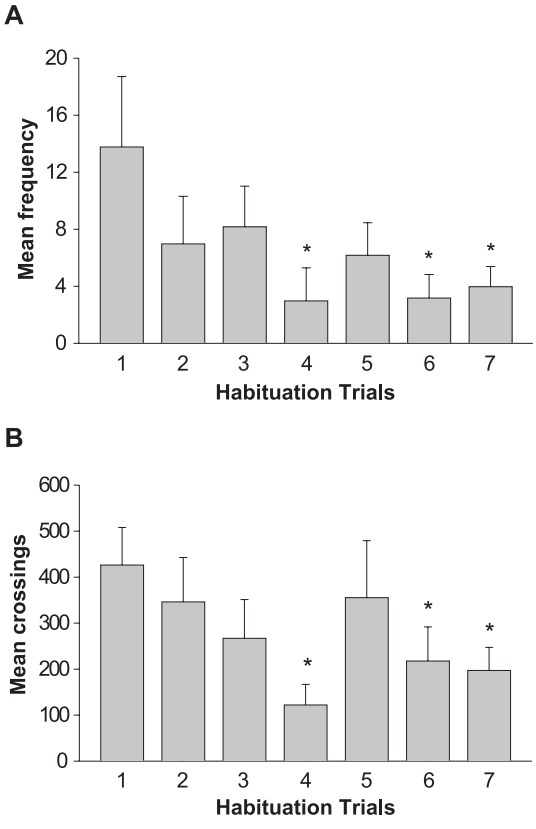

Fig. 2. Effect of each 30-min maze habituation trial, in the absence of the 'predator', on the mean (± S.E.M.) exploratory activity (A) and locomotor activity (B). (*$P < 0.05$ vs. trial 1.)

'predator' were analyzed with two-way ANOVAs for repeated measures (factors: maze section and treatment). Subsequent between- and within-groups analyses were performed using the appropriate error variance terms from the ANOVA summary tables with Duncan's test (habituation trials: trial 1 vs. remaining trials; treatment trials: saline vs. habituation trial 7 and each drug treatment trial). A P value of 0.05 was used for statistical significance.

3. Results

For each behavioral category, the analyzed data were pooled into one group, as no significant gender differences were observed. During the course of the seven maze habituation trials, in the absence of the 'predator', marmosets were found to habituate to the maze environment (Fig. 2). A significant decrease in exploratory [$F(6,28)=3.901$, $P<0.01$] and locomotor activity [$F(6,28)=7.401$, $P<0.001$] were observed during the consecutive seven habituation trials. Post hoc analyses revealed that exploratory and locomotor activity decreased significantly ($P<0.05$) during trials 4, 6 and 7, compared to trial 1. These results indicate that marmosets were fully habituated to the maze environment prior to subsequent trials.

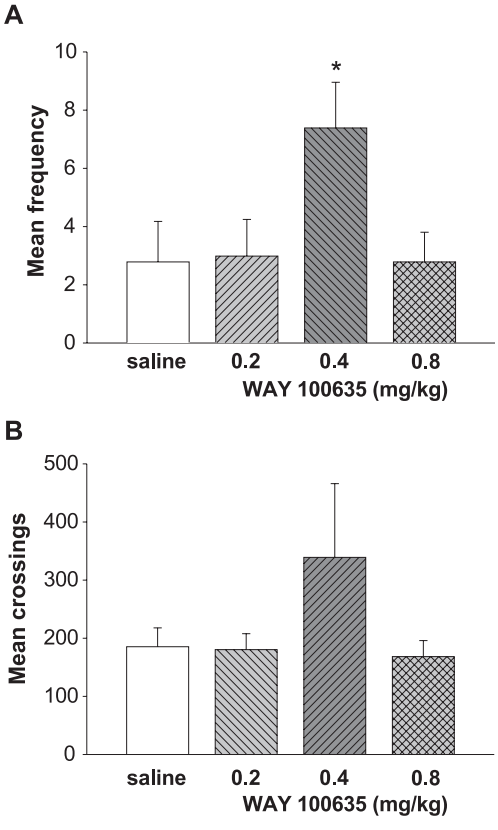

Fig. 3. Effects of WAY 100635 (i.p.) administrations on the mean (± S.E.M.) exploratory (A) and locomotor activity (B) during the 30 min trials in the presence of the 'predator'. (*$P<0.05$ vs. saline.)

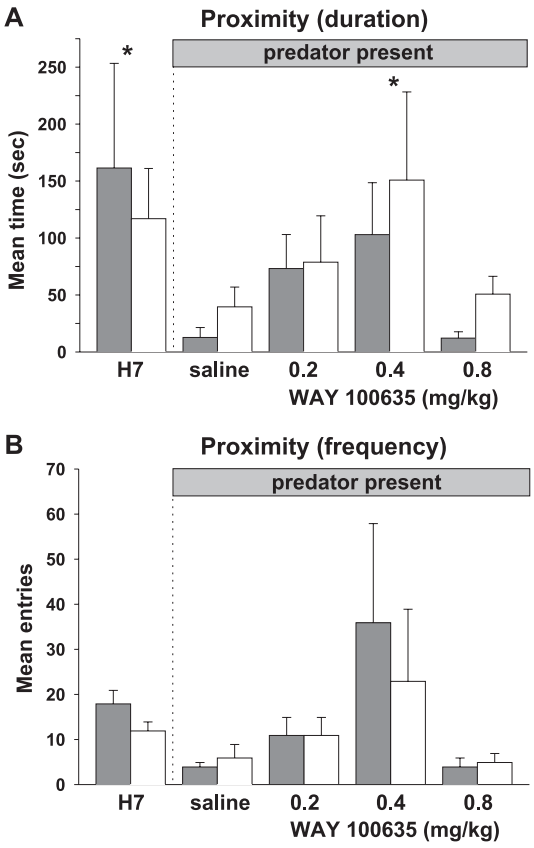

Fig. 4. Effects of WAY 100635 (i.p.) administrations on the mean (± S.E.M.) time spent (A) and frequency (B) in the maze sections closest to (gray bars) and immediately adjacent to (white bars) the 'predator' stimulus location during the 30 min trials. (H7 = habituation trial 7; *$P<0.05$ vs. saline.)

During the treatment trials, when the 'predator' was present, WAY 100635 administration was found to significantly alter exploratory activity [$F(6,28)=4.047$, $P<0.05$; Fig. 3A]. Further analysis indicated that only the dose of 0.4 mg/kg significantly increased this parameter, compared to saline control ($P<0.05$). Notably, analysis of the time spent in the maze sections closest to (S_1/S_2) and immediately adjacent to (S_3/S_4) the 'predator' location (i.e., proximity to 'predator'; Fig. 4A) revealed that the stimulus and treatments significantly influenced this parameter [treatment: $F(4,32)=2.800$, $P<0.05$]. The presence of the 'predator' induced a significant decrease in the duration of proximity (habituation trials 7 vs. saline; $P<0.05$), indicating a fear-induced avoidance of the stimulus. WAY 100635 treatment of 0.4 mg/kg, on the other hand, significantly reversed this fear-induced avoidance behavior ($P<0.05$), relative to saline control. Similarly, analyses of frequency (Fig. 4B) revealed a decrease in proximity to 'predator' (habituation trial 7 vs. saline), whereas 0.4 mg/kg WAY 100635 also reversed the marmosets' fear-induced avoidance, although not significantly [treatment: $F(4,32)=2.430$, $P=0.067$]. Furthermore, marmosets on average spent more time and went more frequently to the adjacent maze section (S_3/S_4)

when the predator was present, compared to the proximal one (S_1/S_2), however, this difference was not found to be significant [duration: $F(1,8)=0.170$, $P=0.680$; frequency: $F(1,8)=0.390$, $P=0.550$]. Maze section vs. treatment interactions were not statistically significant [duration: $F(4,32)=0.330$, $P=0.850$; frequency: $F(4,32)_{4,32}=0.210$, $P=0.920$], and sedation as manifested in decreased locomotion was not observed at any dose of WAY 100635 [$F(3,16)=1.435$, $P=0.281$; Fig. 3B].

4. Discussion

In the Marmoset Predator Confrontation Test–an ethologically based fear/anxiety test in non-human primates (Barros and Tomaz, 2002)–the selective silent 5-HT_{1A} receptor antagonist WAY 100635 altered the animals' behavioral repertoire suggestive of an anxiolytic profile. Consistent with previously findings in this test (Barros and Tomaz, 2002; Barros et al., 2002a), WAY 100635 treatment (0.4 mg/kg) was found to significantly reverse fear-induced avoidance of the maze sections closest to the 'predator', and increase the frequency of maze exploration. Importantly, these results were not influenced by motor impairment. At a similar dose range, WAY 100635 has also failed to modify locomotor activity in rodents (Cao and Rodgers, 1997, 1998; Griebel et al., 1999, 2000). An apparent bell-shaped dose–response curve for the anxiolytic effects was observed, similar to results and dose range found in the different rodent tests of anxiety (Cao and Rodgers, 1997, 1998; Griebel et al., 1999, 2000). Accordingly, WAY 100635 systemically administered at low doses (≤ 0.2 mg/kg) may not yet have attained anxiolytic properties in marmosets. On the other hand, at a higher dose (0.8 mg/kg), an antagonistic action of WAY 100635 and its metabolite, WAY 100634, at α_1-adrenoceptor sites could induce an opposing anxiogenic-like response, counteracting the WAY 100635 anxiolytic effects at postsynaptic 5-HT_{1A} receptors (Cao and Rodgers, 1997). In fact, WAY 100634 demonstrates a high affinity for α_1-adrenoceptors, particularly at high doses, while that of WAY 100635 has been shown to be only moderate to low (Fletcher et al., 1996; Pike et al., 1996). Such a relatively narrow dose–response curve of the observed anxiolytic-like effect is consonant with previous findings in the Marmoset Predator Confrontation Test (Barros et al., 2000, 2001, 2002a, 2002b), albeit not with other primate models (e.g., Kalin et al., 1987; Costall et al., 1992; Cilia and Piper, 1997). This disparity, as also observed among rodent tests of fear/anxiety, may be due to the nature of the response being investigated (i.e., conspecific confrontation vs. social isolation vs. predator stress; e.g., Blanchard et al., 1998). Predatory stress most likely involves different aspects of anxiety, as, for example, conspecific confrontation paradigms do. As such, it may provide a complementary way to assess anxiety-related behaviors, which, however, may have a narrower sensitivity to pharmacological manipulations. To our knowledge, this paradigm is the first attempt to investigate acute predatory stress and its involvement in fear/anxiety responses in primates.

Previous studies in the Marmoset Predator Confrontation Test with the 5-HT_{1A} receptor partial agonist buspirone (Barros et al., 2001) resulted in more significant effects on a wider range of behavioral indicators of anxiety than that with WAY 100635. Both contrary (e.g., Griebel et al., 2000) and similar findings (Cao and Rodgers, 1997; Bell et al., 1999) with rodents, however, indicate that factors aside from inter-species differences should be accountable for this discrepancy. In fact, significant differences between the nature of the response being induced and studied are known to exist, which in turn are thought to be mediated by distinct 5-HT_{1A} receptor mechanisms (Griebel et al., 2000). As a result, differences in the roles of pre- and postsynaptic 5-HT_{1A} receptors in anxiety may account for the WAY 100635 vs. buspirone profiles observed in this test. Accordingly, buspirone derives its anxiolytic properties from an agonistic action at inhibitory somatodendritic 5-HT_{1A} autoreceptors and an antagonistic one at postsynaptic 5-HT_{1A} sites (e.g., Dourish, 1987), of which either or both properties yield a decline in 5-HT neurotransmission. The 5-HT_{1A} receptor antagonist WAY 100635, on the other hand, has a potent and selective antagonistic action at both pre- and postsynaptic 5-HT_{1A} receptor sites (e.g., Fletcher et al., 1996). Therefore, although WAY 100635 can inhibit hippocampal cell firing (Fletcher et al., 1996) and decrease 5-HT concentrations in the hippocampus and nucleus accumbens (Hjorth et al., 1997; Müller et al., 2002a), it can also prevent 5-HT_{1A} receptor-mediated auto-inhibition of the firing frequency of 5-HT neurons in the raphe nuclei (Forster et al., 1995; Fletcher et al., 1996; Fornal et al., 1996; Mundey et al., 1996). These former effects may, in fact, be mediated by suppression of noradrenergic neuron activity in the locus coeruleus (Blier et al., 2001; Szabo and Blier, 2001).

In addition, as both serotonergic compounds studied to date (i.e., buspirone and WAY 100635) are metabolized into compounds known to act on α-adrenoceptors (Caccia et al., 1986; Fletcher et al., 1996; Pike et al., 1996), some caution should be taken when interpreting the involvement of 5-HT_{1A} receptors in the Marmoset Predator Confrontation Test. However, activation of this receptor is expected to induce an anxiogenic-like behavioral response, and the patterns observed with buspirone (Barros et al., 2001) and here, WAY 100635, indicate an anxiolytic-like effect. Accordingly, 5-HT_{1A} receptors are likely to be involved as well, mediating the anxiolytic action of buspirone and WAY 100635. Studies employing more selective 5-HT_{1A} receptor agonist could provide important insights in the involvement of 5-HT_{1A} receptors in this test, as well as the specific role of pre- vs. postsynaptic serotonergic mechanisms.

Nonetheless, direct behavioral testing of the intrinsic pharmacological potential of WAY 100635 in different rodent tests of anxiety (e.g., Cao and Rodgers, 1997;

Griebel et al., 1999, 2000), and now, in the present test with marmosets, has indicated an anxiolytic potential for this antagonist. To our knowledge, this is the first study to report anxiolytic-like effects of the selective 5-HT_{1A} receptor antagonist WAY 100635 in non-human primates. With respect to its potential use as a therapeutic agent for human anxiety disorder, it is interesting that WAY 100635 seems to lack addictive properties, given that it does not affect dopamine levels in the nucleus accumbens (Di Chiara and Imperato, 1988; Müller et al., 2002b).

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (HU 306/23-1 to JPH), by FINATEC (to CT), a NIDA grant R01 DA 05366-14 (to RJC) and by CAPES/DAAD/PROBRAL (137/02 to CT and JPH). MB was a recipient of a post-doctoral fellowship from CAPES, and CT a recipient of a CNPq researcher fellowship (no. 300364/1986-5).

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