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Short communication

The 5-HT_{1A} receptor antagonist robalzotan completely reverses citalopram-induced inhibition of serotonergic cell firing

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Abstract

5-HT_{1A} receptor antagonists have been suggested to increase the efficacy of selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitors in the treatment of depression by enhancing the increase in brain 5-HT induced by 5-HT reuptake blockade. Here, the novel 5-HT_{1A} receptor antagonist robalzotan [(*R*)-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide hydrogen (2*R*, 3*R*) tartrate monohydrate] (12.5, 25, 50, 100 μg/kg, i.v.) was found to completely reverse the acute inhibitory effect of citalopram (300 μg/kg i.v.) or paroxetine (100 μg/kg, i.v.) on the activity of 5-HT neurons in the dorsal raphe nucleus in rats. Robalzotan (5, 50 μg/kg, i.v.) by itself increased the firing rate of the majority of 5-HT cells studied. The present results suggest that robalzotan may indeed augment the increases in 5-HT output induced by selective 5-HT reuptake inhibitors by antagonizing the feedback inhibition of 5-HT cell firing produced by such drugs. Thus, robalzotan may be effective by enhancing the action of selective 5-HT reuptake inhibitors or as monotherapy in the treatment of depression.

Keywords: 5-HT_{1A} receptor antagonist; Antidepressant; Electrophysiology; Dorsal raphe nucleus; 5-HT(5 hydroxytryptamine, serotonin) reuptake inhibitor, selective

1. Introduction

Recently, the hypothesis was advanced that 5-HT_{1A} receptor antagonists may accelerate the onset of antidepressant action of selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitors and, moreover, that such agents also may increase the clinical efficacy of 5-HT reuptake inhibitors (see Artigas et al., 1996). This hypothesis was based on preclinical findings showing that acute administration of 5-HT reuptake inhibitors suppresses the activity of midbrain 5-HT neurons, an effect that is mediated indirectly through stimulation of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei. Thus, the increase in availability of 5-HT in brain caused by acutely administered 5-HT reuptake inhibitors becomes somewhat attenuated. However, during chronic administration with such drugs attenuation of this feedback inhibition occurs due to a desensitization of somatodendritic 5-HT_{1A} autoreceptors, resulting in elevated levels of 5-HT in terminal areas. In an open clinical trial Artigas et al. (1994) reported that pindolol, a partial β-adrenoceptor agonist with 5-HT_{1A} receptor antagonistic properties, appears to shorten the latency in therapeutic effect of selective 5-HT reuptake inhibitors as well as to increase their efficacy in depressed patients. These preliminary findings have, in principle, been replicated by several placebo-controlled studies (see Pérez et al., 1997; Tome et al., 1997; Zanardi et al., 1997).

Preclinical studies have shown that pretreatment with 5-HT_{1A} receptor antagonists such as (*S*)-UH-301 or WAY-100635 indeed enhances the increase in 5-HT concentrations in terminal areas produced by acutely administered 5-HT reuptake inhibitors (Gartside et al., 1995; Arborelius et al., 1996). This effect is in all probability due to the ability of 5-HT_{1A} receptor antagonists to block the acute, inhibitory effect of 5-HT reuptake inhibitors on 5-HT cell firing (Arborelius et al., 1995; Gartside et al., 1995).

Robalzotan (generic name for NAD-299) is a newly synthesized compound with high affinity and selectivity for 5-HT_{1A} receptors (Johansson et al., 1997). For instance, robalzotan has been shown to antagonize both pre- and postsynaptic central effects produced by the 5-HT_{1A}

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receptor agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) such as the decrease in 5-HT synthesis and hypothermia as well as several behavioral effects (Johansson et al., 1997). In view of the recent finding that pindolol possesses intrinsic activity at somatodendritic 5-HT_{1A} autoreceptors (Clifford et al., 1998) robalzotan may represent a novel and full 5-HT_{1A} receptor antagonist to be used in combination with a selective 5-HT reuptake inhibitor in the treatment of depression. Thus, the aim of the present study was to investigate if robalzotan can reverse the acute, inhibitory effect produced by the selective 5-HT reuptake inhibitors citalopram or paroxetine on the activity of 5-HT neurons in the dorsal raphe nucleus using single cell recording techniques in anaesthetized rats. For comparison, the effects of the selective 5-HT_{1A} receptor antagonist WAY-100635 (Fletcher et al., 1996) in combination with citalopram were also studied.

2. Materials and methods

2.1. Single cell recording of 5-HT cells in the dorsal raphe nucleus

The electrophysiological methods used have previously been described in detail (Arborelius et al., 1995) and have been approved by the Stockholm South Committee on Ethics of Animal Experimentation. Briefly, male Sprague–Dawley rats weighing between 300 and 600 g (B&K Universal, Sollentuna, Sweden) were anaesthetized with chloral hydrate (400 mg/kg, i.p.). Extracellular recording electrodes were pulled (Narishige vertical puller, Tokyo, Japan) from glass capillaries (Clark Electromedical Instruments, Reading, UK) and filled with 2% Pontamine Sky Blue in 0.5 M sodium acetate. Coordinates, for the dorsal raphe nucleus were 0.8–1.2 mm anterior to lambda and 0 ± 0.1 mm lateral to the midline. Presumed 5-HT neurons were found 5.0–6.0 mm from brain surface and recordings were made from one cell in each animal.

The discriminated action potentials were collected and analyzed by the Spike2 program (Cambridge Electronic Design, Cambridge, UK). All drugs, except for the anaesthetic, were administered i.v. and drug effects were assessed by comparison of the mean firing rate during 1.5 min before drug administration (baseline values) to the mean firing rate during the same time period at maximal drug effect in the same cell. The 5-HT_{1A} receptor antagonist was administered 3–5 min after administration of citalopram or paroxetine.

2.2. Drugs

Robalzotan [(*R*)-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide hydrogen (2*R*, 3*R*) tartrate monohydrate], WAY-100635 (*N*-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride) and paroxetine hydrochloride were provided by Astra Arcus (Södertälje, Sweden). Citalopram hydrobromide was kindly supplied by Lundbeck (Copenhagen, Denmark). (+)-(*R*)-8-hydroxy-2-(dipropylamino)-tetralin HCl [(*R*)-8-OH-DPAT] was synthesized at the Department of Organic Pharmaceutical Chemistry, Uppsala University, Sweden. All drugs were dissolved in 0.9% NaCl, except paroxetine which was dissolved in 5.5% glucose.

2.3. Statistics

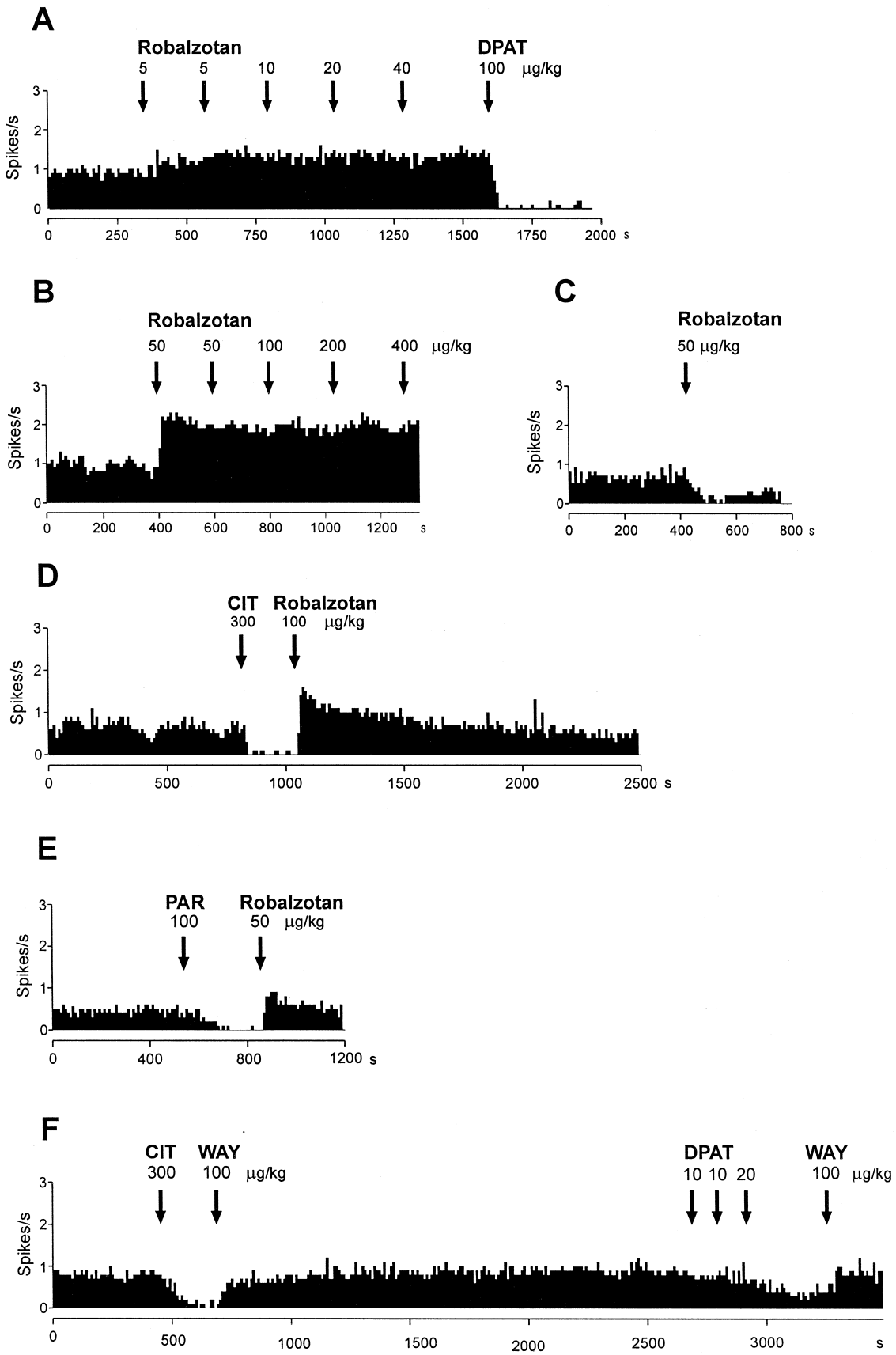
Firing rate values are presented as means ± S.E.M. The effects of robalzotan alone were analyzed statistically by paired *t*-test. The effects of the 5-HT_{1A} receptor antagonists administered after citalopram or paroxetine were analyzed by one-way repeated measures analysis of variance (ANOVA) followed by Tukey test. A two-tailed *P* value less then 0.05 was considered significant.

3. Results

The effect of 0.1 ml of vehicle (5.5% of glucose in the paroxetine experiments and saline in all the other experiments) was studied in all 5-HT cells before administration of the drugs. Since vehicle did not affect the spontaneous activity (data not shown) the firing rate after vehicle was considered as baseline.

Administration of 1 μg/kg of robalzotan did not affect the firing rate of 5-HT cells in the dorsal raphe nucleus (data not shown). At a higher dose, 5 μg/kg, robalzotan significantly ($P < 0.01$) increased the activity of such neurons (Fig. 1A) from 1.35 ± 0.13 Hz to 1.71 ± 0.15 Hz ($n = 7$). However, at 50 μg/kg robalzotan produced variable effects on dorsal raphe 5-HT cells, i.e., the activity of five out of seven cells studied was increased (Fig. 1B) from 0.71 ± 0.10 Hz to 1.31 ± 0.28 Hz, whereas the activ-

Fig. 1. (A–C) Integrated firing rate histograms of three single 5-HT neurons in the dorsal raphe nucleus showing the effect of robalzotan. (A) A typical 5-HT neuron responding with an increased firing rate after 5 μg/kg i.v. of robalzotan. The activity of this neuron was also completely inhibited by (*R*)-8-OH-DPAT (DPAT; 100 μg/kg, i.v.). (B) Showing a 5-HT neuron that responded with an increased activity after administration of 50 μg/kg (i.v.) of robalzotan. (C) Showing a 5-HT neuron that responded with a decrease in firing rate after 50 μg/kg (i.v.) of robalzotan. (D–F) Integrated firing rate histograms of three single 5-HT neurons in the dorsal raphe nucleus showing the effect of acute administration of citalopram (CIT; D, F) or paroxetine (PAR; E) and the subsequent reversal of this effect by robalzotan (D, E) or WAY-100635 (WAY; F). Note that the inhibitory effect of (*R*)-8-OH-DPAT (DPAT) was also reversed by WAY-100635 (WAY) as shown in (F).


A
Robalzotan
5 5 10 20 40
DPAT
100 µg/kg
Spikes/s
0 250 500 750 1000 1250 1500 1750 2000 s
B
Robalzotan
50 50 100 200 400 µg/kg
Spikes/s
0 200 400 600 800 1000 1200 s
C
Robalzotan
50 µg/kg
Spikes/s
0 200 400 600 800 s
D
CIT
300
Robalzotan
100 µg/kg
Spikes/s
0 500 1000 1500 2000 2500 s
E
PAR
100
Robalzotan
50 µg/kg
Spikes/s
0 400 800 1200 s
F
CIT
300
WAY
100 µg/kg
DPAT
10 10 20
WAY
100 µg/kg
Spikes/s
0 500 1000 1500 2000 2500 3000 s

ity of the other two cells was decreased (Fig. 1C) from 0.68 ± 0.01 to 0.36 ± 0.26 Hz. Thus, the overall effect of 50 μg/kg of robalzotan (1.04 ± 0.27 Hz) was not significant from baseline values (0.70 ± 0.07 Hz; $n = 7$). As shown in Figs. 1A and B administration of cumulative, increasing doses of robalzotan (5–100 μg/kg or 50–400 μg/kg) did not further affect the firing rate of 5-HT neurons.

Administration of 300 μg/kg of citalopram significantly decreased the activity of 5-HT cells in the dorsal raphe nucleus (Figs. 1, 2) in agreement with our previous data (Arborelius et al., 1995). Robalzotan (12.5, 25, 50 or 100 μg/kg), administered 3–5 min after citalopram, potently and consistently reversed the inhibitory effect of citalopram (Figs. 1D, 2A), although the inhibition of one cell was not reversed by the lowest dose of robalzotan

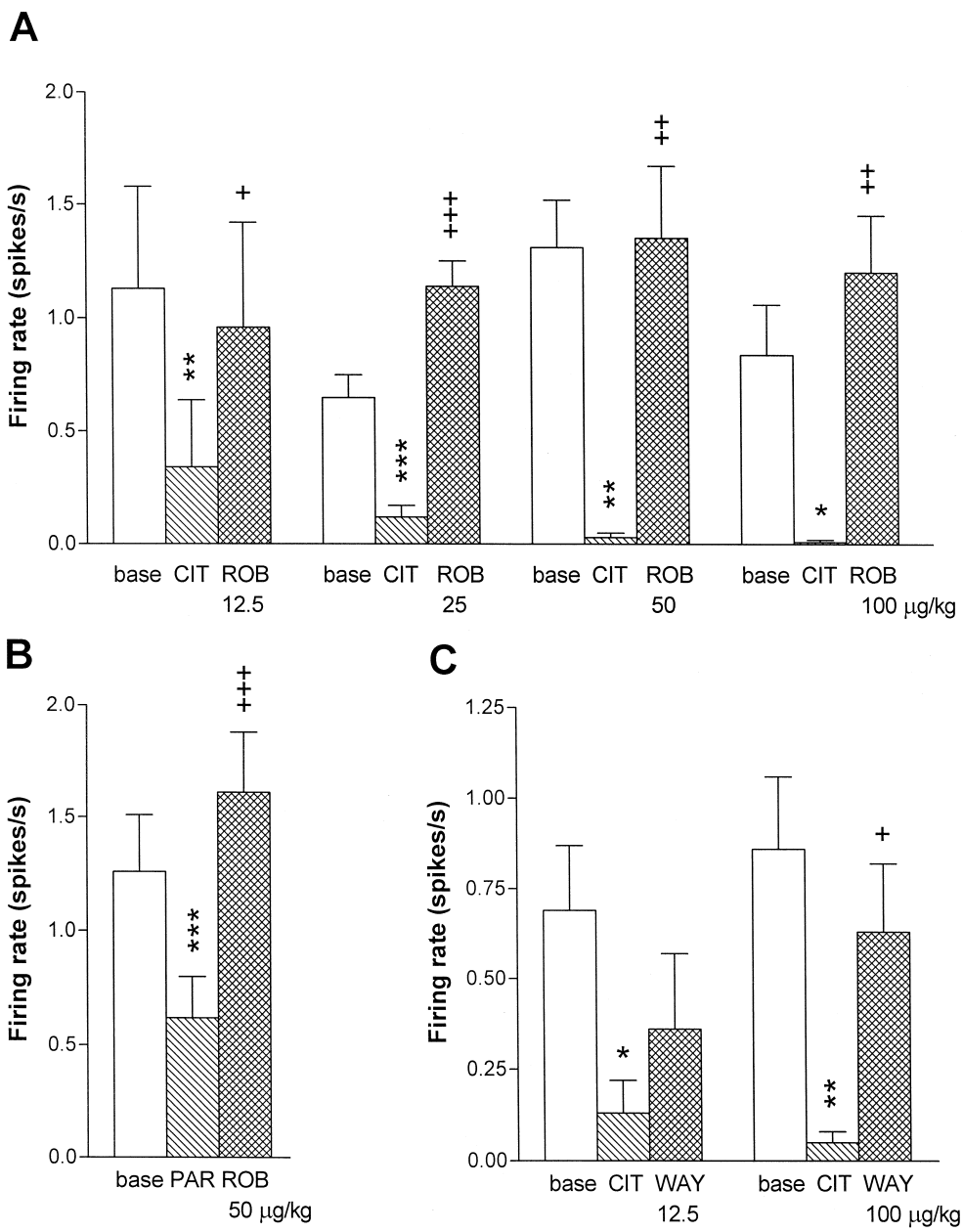


Fig. 2. (A) Graph illustrating the effects of citalopram (CIT; 300 μg/kg, i.v.) alone and after different doses of robalzotan (ROB; 12.5, 25, 50 or 100 μg/kg, i.v.; $n = 5$ in all four groups) on 5-HT cell firing in the dorsal raphe nucleus. (B) Graph illustrating the effect of paroxetine (PAR; 100 μg/kg, i.v.) alone and after robalzotan (ROB; 50 μg/kg, i.v.; $n = 5$) on dorsal raphe cell firing. (C) Graph illustrating the effect of CIT alone and after WAY-100635 (WAY; 12.5 or 100 μg/kg; $n = 5$–6) on the firing rate of 5-HT cells in the dorsal raphe nucleus. Each bar represents the mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ the effect of citalopram (CIT) or paroxetine (PAR) compared with baseline (base) values. $+P < 0.05$, $++P < 0.01$, $+++P < 0.001$ the effect of ROB or WAY compared with the effect of CIT or PAR.

(12.5 μg/kg). Robalzotan (50 μg/kg) could also reverse the acute inhibitory effect of paroxetine (100 μg/kg) on 5-HT cell firing (Figs. 1E, 2B). As expected administration of WAY-100635 (100 μg/kg) significantly reversed the inhibitory effect of citalopram on dorsal raphe 5-HT cell firing (Figs. 1F, 2C), although in two of the six cells studies WAY-100635 failed to do so. A lower dose, 12.5 μg/kg, of WAY-100635 reversed the citalopram-induced inhibition only in two out of five cells studied. Thus, the overall effect of the low dose of WAY-100635 was not statistically significant (Fig. 2C).

4. Discussion

In the present study, the acute, inhibitory effect of both citalopram and paroxetine on the activity of 5-HT cells in the dorsal raphe nucleus was instantly and completely reversed by administration of robalzotan. This is in agreement with previous studies showing that 5-HT_{1A} receptor antagonists block the inhibitory effect of selective 5-HT reuptake inhibitors on central 5-HT neurons (Arborelius et al., 1995; Gartside et al., 1995). Since the suppressant effect of 5-HT reuptake inhibitors on central 5-HT neurons is most likely indirectly mediated through increased stimulation of somatodendritic 5-HT_{1A} autoreceptors, the present findings provide additional evidence that robalzotan acts as a potent antagonist at central 5-HT_{1A} receptors. This notion is further supported by a recent study where robalzotan was found to potently antagonize the inhibitory effect of 8-OH-DPAT on 5-HT cell firing in the dorsal raphe nucleus (Martin et al., 1999).

In agreement with a previous study, administration of 100 μg/kg of WAY-100635 reversed the inhibitory effect of a selective 5-HT reuptake inhibitor on 5-HT neuronal activity in the majority of the cells studied (Gartside et al., 1995), whereas a much lower dose of WAY-100635 (12.5 μg/kg) reversed the suppressant effect of citalopram only in two out of five neurons studied. In contrast, administration of the same dose of robalzotan reversed the citalopram-induced inhibition of all but one of the 5-HT cells studied. The present findings may indicate that robalzotan is a more potent antagonist at somatodendritic 5-HT_{1A} autoreceptors than WAY-100635. Alternatively, the difference in efficacy between the compounds may be related to a possible existence of different types of 5-HT cells that respond differentially. Indeed, previous studies have shown that the inhibitory effect of selective 5-HT reuptake inhibitors on 5-HT cell firing could not be reversed in all cells by 5-HT_{1A} receptor antagonists (see Arborelius et al., 1995; Hajós et al., 1995).

Interestingly, robalzotan when given alone increased the firing rate of the majority of 5-HT cells tested. However, after the higher dose of robalzotan (50 μg/kg) the activity of a few 5-HT neurons were slightly inhibited. This is in agreement with previous studies in anaesthetized rats showing that administration of other 5-HT_{1A} receptor antagonists increases the activity of some 5-HT cells whereas that of other cells is inhibited (Arborelius et al., 1994; Gartside et al., 1995). The excitatory effect of robalzotan is probably due to an antagonism of a tonically active, inhibitory influence by endogenous 5-HT, mediated through somatodendritic 5-HT_{1A} autoreceptors. A recent study also found that robalzotan increases 5-HT synthesis in the nuclues accumbens (Ahlenius et al., 1998) which taken together with the present finding suggests that robalzotan alone may stimulate central serotonergic systems. However, the present findings are somewhat at variance with a previous study where robalzotan in low doses did not produce any significant increase in the firing rate of 5-HT cells in the dorsal raphe nucleus, and at higher doses produced a significant decrease (Martin et al., 1999). The reason for this discrepancy is not clear but may be related to differences in the administration of the drug. Thus, in the present study the effects of a single dose of robalzotan was studied whereas in the study by Martin et al. the drug was given in increasing, cumulative doses. Actually, we did not observe any effect of a cumulative dose of 4 μg/kg of robalzotan (data not shown) but found a significant increase in firing rate after a single dose of 5 μg/kg.

The inhibitory effect of the higher dose of robalzotan observed in the present study on some 5-HT cells may be related to other properties of the drug such as its affinity for α_1-adrenoceptors (Johansson et al., 1997). Indeed, Martin et al. (1999) found that the inhibitory effect of 20 μg/kg of robalzotan on 5-HT cell firing was completely reversed by d-amphetamine which increases the noradrenergic tone, suggesting that robalzotan at the high dose (50 μg/kg) used in the present study also blocks excitatory α_1-adrenoceptors.

It has been suggested that a 5-HT_{1A} receptor antagonist may enhance the antidepressant action of selective 5-HT reuptake inhibitors by blocking the suppression of 5-HT cell firing induced by such drugs and thereby produce a greater increase in extracellular concentrations of 5-HT in brain than a selective 5-HT reuptake inhibitor alone. Moreover, several clinical studies have shown that the combination of a selective 5-HT reuptake inhibitor with pindolol produces a faster onset in clinical effect as well as a higher efficacy than treatment with a selective 5-HT reuptake inhibitor alone (see Introduction). However, preclinical studies have shown that pindolol decreases the activity of 5-HT cells in the dorsal raphe nucleus, an effect that could be reversed by WAY-100635 (Clifford et al., 1998; Arborelius et al., in preparation). Moreover, according to recent studies pindolol cannot reverse the acute inhibitory effect of a selective 5-HT reuptake inhibitor on 5-HT cell firing and blocks this effect only at high doses suggesting that pindolol possesses only weak antagonistic properties at 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (Arborelius et al., in preparation; Metzler et al., 1997). On the other hand, robalzotan as shown by the present study,

can completely reverse the acute inhibitory effect of selective 5-HT reuptake inhibitors, and preliminary results suggest that robalzotan also markedly enhances the acute increase in 5-HT concentrations in the frontal cortex produced by citalopram (Hjorth, 1998). Therefore, it would be of considerable interest to study the clinical effect of robalzotan, which appears to be a selective and silent 5-HT_{1A} receptor antagonist, in combination with a selective 5-HT reuptake inhibitor in the treatment of depression. Robalzotan may also represent an interesting drug candidate for monotherapy in depression, since it appears to stimulate brain serotonergic systems even when given alone.

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