Sustained pharmacological blockade of NK₁ substance P receptors causes functional desensitization of dorsal raphe 5-HT₁A autoreceptors in mice

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Abstract

Antagonists at NK₁ substance P receptors have demonstrated similar antidepressant properties in both animal paradigms and in human as selective serotonin reuptake inhibitors (SSRIs) that induce desensitization of 5-HT₁A autoreceptors within the dorsal raphe nucleus (DRN). We investigated whether this receptor adaptation also occurs upon NK₁ receptor blockade. C57B/L6J mice were treated for 21 days with the selective NK₁ receptor antagonist GR 205171 (10 mg/kg daily) through subcutaneously implanted osmotic mini pumps, and DRN 5-HT₁A autoreceptor functioning was assessed using various approaches. Recording of DRN serotonergic neurons in brainstem slices showed that GR 205171 treatment reduced (by ~1.5 fold) the potency of the 5-HT₁A receptor agonist, ipsapirone, to inhibit cell firing. In parallel, the 5-HT₁A autoreceptor-mediated [³⁵S]GTP-γ-S binding induced by 5-carboxamidotryptamine onto the DRN in brainstem sections was significantly decreased in GR 205171-treated mice. In vivo microdialysis showed that the cortical 5-HT overflow caused by acute injection of the SSRI paroxetine (1 mg/kg) was twice as high in GR 205171-treated as in vehicle-treated controls. In the DRN, basal 5-HT outflow was significantly enhanced by GR 205171 treatment. These data supported the hypothesis that chronic NK₁ receptor blockade induces a functional desensitization of 5-HT₁A autoreceptors similar to that observed with SSRIs.

Keywords: 5-HT₁A autoreceptor, desensitization, dorsal raphe nucleus, NK₁ receptors, substance P receptor antagonists.


A large body of evidence supports the idea that a deficit in central serotonin (5-hydroxytryptamine, 5-HT) neurotransmission is associated with depression (Coppen et al. 1973; Asberg et al. 1984; Delgado et al. 1990). In line with this concept, effective treatment of the disease is achieved with selective serotonin reuptake inhibitors (SSRIs) that enhance central 5-HT neurotransmission (Bel and Artigas 1993; Kreiss and Lucki 1995), mainly through a functional desensitization of somatodendritic 5-HT₁A autoreceptors located on dendrites and somata of 5-HT neurons within the dorsal raphe nucleus (DRN) (Blier and De Montigny 1983; Le Poul et al. 2000). Because 5-HT₁A autoreceptor activation inhibits 5-HT neuron firing (Aghajanian et al. 1987), this desensitization may promote 5-HT neurotransmission, thereby representing an adaptive change relevant to the therapeutic effect of SSRIs.

Although SSRIs are effective in most depressive episodes, they do not alleviate symptoms in 30% of depressed patients and some adverse effects have been reported after long-term treatment with these drugs (Stahl 1998). In addition, the therapeutic efficacy of SSRIs is blunted by the long delay in

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Abbreviations used: aCSF, artificial cerebrospinal fluid; AUC, area under curve; DRN, dorsal raphe nucleus; 5-HT, serotonin; NS, not significant; n.s., non-specific; OD, optical density; PLSD, protected least significance difference; SSRIs, selective serotonin reuptake inhibitors.
clinical benefit. Accordingly, different strategies are under way in order to develop novel antidepressant therapies. Recent clinical and preclinical studies have pointed out the potential therapeutic action of substance P receptor antagonists (NK₁ receptor antagonists) in major depressive disorders (Rupniak 2002a,b). The implication of substance P neurotransmission in mood disorders had been previously evoked by Rimon et al. (1984), who reported an increase in substance P levels in the cerebrospinal fluid of depressed patients. Substance P is a neuropeptide which belongs to the tachykinin family. Tachykinins bind to three distinct receptors designated NK₁, NK₂ and NK₃. In rodent brain, substance P actions are preferentially mediated via the NK₁ receptors, although a mediation via NK₂ and NK₃ receptors is also possible as substance P has some affinity for those receptors (Saria 1999; Strand 1999). In various animal species, pharmacological blockade of NK₁ receptors has been shown to attenuate behavioural and neurochemical responses to stress (Ebner et al. 2004; Hutson et al. 2004), to reduce ultrasonic vocalization in pups isolated from their mother (Kramer et al. 1998), to decrease aggressiveness (Shaikh et al. 1993), to restore responsiveness to rewarding stimuli in rats subjected to chronic mild stress (Papp et al. 2000) and to reduce immobility time in the forced swimming test (Zocchi et al. 2003).

In humans, a clear-cut antidepressant action, similar to that of the SSRI paroxetine, was reported for the NK₁ receptor antagonists MK-869 and L-759274 by Kramer et al. (1998, 2004). However, the precise neurobiological mechanisms underlying the antidepressant action of these NK₁ receptor antagonists have yet to be elucidated.

As emphasized above, DRN 5-HT₁₄ autoreceptors appear to be critically involved in the antidepressant action of SSRIs. Accordingly, whether NK₁ receptor antagonists also act, albeit indirectly, through 5-HT₁₄ autoreceptors is an important question to be addressed. Recently, this issue was assessed in NK₁ receptor knock-out mice in which a marked functional desensitization of 5-HT₁₄ autoreceptors was observed in the DRN (Froger et al. 2001). Whether or not sustained pharmacological blockade of NK₁ receptors could mimic the effect of NK₁ receptor gene knock-out has now been investigated in mice. For this purpose, wild-type C57BL/6J mice were chronically treated with GR 205171, a selective and brain penetrating NK₁ receptor antagonist (Gardner et al. 1996), and electrophysiological, autoradiographic and biochemical approaches were used to assess possible changes in 5-HT₁₄ receptor functioning caused by the treatment.

Materials and methods

Animals

Experiments were performed on male C57BL/6J mice (CER Janvier, Le Genest-St Isle, France) weighing 20–25 g (8–10 weeks old) and housed six per cage under standard conditions (12-h light/dark cycle, 22 ± 1°C ambient temperature, 60% relative humidity, food and water ad libitum).

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive no. 87-848, 19 October 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions no. 75-116 to MH, no. 75-977 to LL and no. 92-196 to AMG).

Pharmacological treatments

Acute treatments

Mice were injected subcutaneously with either GR 205171 (10 mg/kg) or its vehicle (0.9% NaCl), and were decapitated 60 min later. Brains were rapidly removed in order to proceed with electrophysiological experiments.

For microdialysis experiments, mice received a challenge dose of paroxetine (1 mg/kg, i.p.) or a combined treatment with paroxetine plus WAY106635 (0.5 mg/kg, s.c.). Control animals were injected with the vehicle (saline) through the same routes of administration.

Chronic treatments

Mice were treated for 21 days with the NK₁ receptor antagonist GR 205171. The compound was dissolved in 0.9% NaCl/dimethylsulfoxide (80/20) at a concentration of 20 mg/mL to produce an average dose of 10 mg/kg/day. Then, 100 μL of this solution were filled into the osmotic mini pump (Alzet®, 1007D, Charles River Laboratories Inc., Wilmington, MA, USA) ensuring constant delivery (0.5 μL/h) for 7 days. The mini pump was implanted subcutaneously on the back under light anaesthesia with ether; this procedure was repeated two times for the 3-week treatment of each animal. Control mice were implanted with mini pumps filled with vehicle only. Control and treated mice were decapitated 24 h after removal of the third mini pump, and brains were rapidly taken out to perform electrophysiological and [35S]GTP-γ-S binding experiments (see below). For microdialysis experiments, the third mini pump was removed on day 21, just before implantation of the microdialysis probe either in the frontal cortex or in the DRN. Animals were then returned to their home cage for recovery until the start of dialysate sample collection.

Electrophysiological experiments

Immediately after removal from the skull, the brain was immersed in an ice-cold artificial cerebrospinal fluid (aCSF1) of the following composition (mM): NaCl 126, KCl 3.5, NaH₂PO₄ 1.2, MgCl₂ 1.3, CaCl₂ 2.0, NaHCO₃ 25, n-glucose 11, continuously bubbled with carbogen (95% O₂/5% CO₂) to maintain pH value at 7.3.

A block of tissue containing the DRN was cut into sections (400-μm thick) in the same ice-cold aCSF1 using a vibratome. Brain stem slices were immediately immersed in oxygenated aCSF1 at room temperature (22°C). A single slice was then placed on a nylon mesh, completely submerged in the recording chamber and continuously superfused with oxygenated aCSF1 (34°C) at a constant flow rate of 2–3 mL/min (Haj-Dahmane et al. 1991).

Extracellular recordings of the firing of DRN serotonergic neurons were made using glass microelectrodes filled with 2 M NaCl (12–15 MΩ). Cells were identified as 5-HT neurons according...
Immediately after removal from the skull, brains were frozen by Hamon 1997) at four different concentrations (10

Concentric dialysis probes were made of cuprophan fibres and set up as described previously (Malagie et al. 1996). Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. The probes were implanted into either the frontal cortex or the DRN (active length: 1.6 × 0.3 mm outer diameter and 1.0 × 0.3 mm outer diameter, respectively) according to the ‘Mouse brains’ atlas of Hof et al. (2000). Stereotaxic coordinates from bregma were (in mm) for the frontal cortex: anterior = +1.6, lateral = +1.3, ventral = −1.6, and for the DRN: anterior = −4.5, lateral = 0, ventral = −3.0. Animals were allowed to recover from surgery overnight and the next day, i.e. ~20 h after the surgery, continuous perfusion of the probe with aCSF1 (composition in mM: NaCl 147, KCl 3.5, CaCl2 1.26, MgCl2 1.20, NaH2PO4 1.0, pH 7.4) started at a flow rate of 1.5 and 0.5 μL/min in the frontal cortex and the DRN, respectively, using a CMA/100 pump (Carnegie Medicine, Stockholm, Sweden). Dialysates were collected in Eppendorf tubes for the measurement of extracellular levels of 5-HT ([5-HT]ext) using a high-performance liquid chromatography (HPLC) system (XL-ODS, 4.6 × 75 mm, particle size 3 μm; Beckman, Roissy-Charles-de-Gaulle, France) coupled to an amperometric detector (HP1049A, Hewlett-Packard, Les Ulis, France). The dialysate fractions were collected every 30 min in the DRN, and every 15 min in the frontal cortex, so as to adapt to the detection limit of our HPLC system and to allow reliable quantification of 5-HT accumulated per sample. The basal value of [5-HT]ext was calculated as the mean ± SEM of the first four samples collected before systemic administration of drugs. Extracellular 5-HT levels were not corrected for probe recovery and/or differences in probe length. The limit of sensitivity for the quantification of [5-HT]ext was ~ 0.5 fmol per sample (signal-to-noise ratio = 2).

At the end of microdialysis experiments, mice were killed by cervical dislocation, brains were removed and stored at −40°C. To determine the exact probe implantation into the frontal cortex or the DRN, brains were placed in a Kryomat apparatus and kept at −25°C. Coronal frozen sections were cut serially at 40 μm intervals (Guiard et al. 2004). Each section was photographed using a digital camera, and brain regions were identified according to Hof et al. (2000). Only mice with probes confined to either the frontal cortex or the DRN were used for subsequent data analyses.

### Statistical analyses

All data are given as means ± SEM. Extracellular recordings and [35S]GTP-γ-S binding were analyzed by two-way ANOVA followed by Bonferroni post-hoc tests. Unpaired two-tailed Student’s t-test was used also to compare the treated groups with their control.

Microdialysis results were analyzed by the computer software StatView 5.0 (Abacus Concepts Inc., Berkeley, CA, USA), used to compare ‘area under the curve’ (AUC; mean ± SEM) values expressed as percentage of basal [5-HT]ext levels. These AUC values were calculated as the amount of 5-HT outflow collected during the 0–180-min period after the administration of a challenge dose of saline, paroxetine alone or paroxetine plus WAY 100635. For each brain region, a two-way ANOVA on AUC values was performed with the chronic drug treatment (vehicle or GR 205171 at 10 mg/kg/day for 21 days) and the single challenge injection performed on day 21 (saline, paroxetine alone or paroxetine plus WAY 100635) as main factors, followed by Fisher protected least significance difference (PLSD) post-hoc test. Statistical significance was set at p < 0.05.
Drugs
GR 205171 [2-methoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-(2-phenylpiperidin-3-yl)-amine] and paroxetine were generously given by GlaxoSmithKline (Harlow, UK). \[^{35}S\]GTP-\(\gamma\)-S was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Other compounds were: N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-N-(2-pyridinyl)-cyclohexanecarboxamide (WAY 100635, Wyeth-Ayerst, Princeton, NJ, USA), GDP dilithium salt (Boehringer-Mannheim, Meylan, France), DPCPX and 5-CT (Research Biochemicals Inc., Natick, MA, USA), ipsapirone (Troponwerke Bayer, Cologne, Germany).

Results

Effects of the NK\(_1\) antagonist, GR 205171, on the electrical activity of DRN 5-HT neurons

In vitro application of GR 205171

DRN serotonergic neurons recorded in vitro in brainstem slices from C57BL/6J mice displayed a characteristic slow (1.74 ± 0.32 spikes/s, mean ± SEM, \(n = 6\)) and regular pattern of discharge (Fig. 1), as previously described for other mouse strains (Lanfumey et al. 1999).

The typical example illustrated in Fig. 1 shows that superfusion of brainstem slices with increasing concentrations of GR 205171 (10\(^{-9}\)–10\(^{-5}\) M) did not significantly modify the firing rate of DRN serotonergic neurons. In contrast, the 5-HT\(_{1A}\) receptor agonist ipsapirone produced a concentration-dependent inhibition of the electrophysiological activity of the same neurons (Fig. 1).

Acute in vivo administration of GR 205171

Neither the frequency nor the pattern of the discharge of DRN serotonergic neurons were significantly altered in mice that had been given GR 205171 (10 mg/kg s.c.) or saline 60 min before death compared with naive, untreated, mice (not shown). Furthermore, the dose–response curve of ipsapirone-induced inhibition was similar in GR 205171-treated mice (EC\(_{50}\) = 46.8 ± 5.0 nm, mean ± SEM, \(n = 6\)) and in paired saline-treated controls (EC\(_{50}\) = 36.1 ± 3.3 nm, mean ± SEM, \(n = 6\); \(F_{1,42} = 3.04,\ NS\); Fig. 2). In both groups, complete inhibition of DRN 5-HT neuron firing was obtained by tissue superfusion with 100 nm ipsapirone (Figs. 2a and b).

Chronic administration of GR 205171

Chronic treatment with GR 205171 (10 mg/kg/day s.c. for 21 days), such as that noted after acute administration, did not alter the electrophysiological activity of DRN 5-HT neurons recorded in brainstem slices. The firing rate of these neurons was similar in mice that had been treated for 3 weeks with either GR 205171 (1.80 ± 0.20 spikes/s, \(n = 15\)) or saline (1.80 ± 0.10 spikes/s, \(n = 21\)) and in the same range as that of naive, untreated, animals (see above).

Although ipsapirone caused a concentration-dependent inhibition of the firing of DRN serotonergic neurons in both saline- and GR 205171-treated mice (Figs. 3a and b), this effect appeared significantly less pronounced in the latter group. Indeed, the concentration–response curve of ipsapirone in GR 205171-treated mice was shifted to the right compared with that for paired control mice \((F_{1,209} = 110.11, p < 0.0001)\), with an EC\(_{50}\) value of the 5-HT\(_{1A}\) receptor agonist ∼1.5 times higher in GR 205171-treated animals (57.5 ± 8.3 nm, \(n = 15\)) than in the saline-treated group (37.0 ± 4.4 nm, \(n = 21\), \(p < 0.05\)) (Fig. 3b). Furthermore, complete inhibition of DRN 5-HT neuron firing required only 100 nm ipsapirone in the latter group, but up to 300 nm of the drug in GR 205171-treated mice (Fig. 3a).

Effects of chronic administration of GR 205171 on 5-HT\(_{1A}\) receptor-mediated \[^{35}S\]GTP-\(\gamma\)-S binding

Because electrophysiological experiments showed the occurrence of DRN 5-HT\(_{1A}\) autoreceptor desensitization in mice that had been treated for 3 weeks with GR 205171 (10 mg/kg/day s.c.), further investigations were made using autoradiographic measurement of \[^{35}S\]GTP-\(\gamma\)-S binding in order to assess G-protein coupling of this receptor. Under basal conditions, i.e. in the absence of 5-CT, \[^{35}S\]GTP-\(\gamma\)-S labelling within the DRN did not differ between GR 205171-treated mice (OD 9.6 ± 0.5, \(n = 5\)) and paired vehicle-treated controls (9.2 ± 0.8, \(n = 4\); Fig. 4a). In both groups, 5-CT induced a concentration-dependent increase in
S-GTP-c labelling, which could be completely prevented by the selective 5-HT1A receptor antagonist WAY 100635 (at 10 μM; Fig. 4a). However, the 5-CT-induced increase in [35S]GTP-c labelling was significantly less in the GR 205171-treated group ($F_{1,34} = 264.62, p < 0.0001$). Thus, the concentration–response curve of 5-CT-evoked [35S]GTP-c labelling in GR 205171-treated mice ($EC_{50} = 47.4 ± 16.1 \text{ nM}, n = 5$) was shifted to the right compared with that for vehicle-treated mice ($EC_{50} = 13.6 ± 1.2 \text{ nM}, n = 4; p < 0.05$; Table 1; Fig. 4b). Furthermore, the maximal [35S]GTP-c labelling ($E_{\text{max}}$) obtained with 1–10 μM 5-CT was significantly lower in GR 205171-treated mice than in paired controls (Fig. 4b; Table 1).

Fig. 2 Effects of ipsapirone on the firing of DRN 5-HT neurons in mice injected acutely with GR 205171 (10 mg/kg s.c.) or its vehicle. Electrophysiological recordings were performed in brainstem slices from mice decapitated 60 min after the treatment. (a) Integrated firing rate histograms (in spikes per 10 s) showing ipsapirone-induced inhibition of 5-HT neuron firing in both a GR 205171- and a vehicle-injected mouse (bottom and top recordings, respectively). (b) Concentration–response curves of ipsapirone-induced inhibition of the firing of DRN 5-HT neurons in brainstem slices from GR 205171- and vehicle-injected mice. Ipsapirone-induced inhibition is expressed as a percentage of the baseline firing rate. Each point is the mean ± SEM of data obtained from five to six individual cells. The dotted lines illustrate the $EC_{50}$ values of ipsapirone (abscissa) in GR 205171- and vehicle-injected mice.

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Fig. 3 Effects of ipsapirone on the firing of DRN 5-HT neurons in mice after a 3-week treatment with GR 205171 (10 mg/kg/d s.c.) or its vehicle. Electrophysiological recordings were performed in brainstem slices from mice decapitated 24 h after removal of osmotic mini pump delivering GR 205171 or the vehicle. (a) Integrated firing rate histograms (in spikes per 10 s) showing ipsapirone-induced inhibition of 5-HT neuron firing in both a GR 205171- and a vehicle-treated mouse (bottom and top recordings, respectively). (b) Concentration–response curves of ipsapirone-induced inhibition of the firing of DRN 5-HT neurons in brainstem slices from GR 205171- and vehicle-treated mice. Ipsapirone-induced inhibition is expressed as a percentage of the baseline firing rate. Each point is the mean ± SEM of data obtained from 15 to 21 individual cells. The dotted lines illustrate the $EC_{50}$ values of ipsapirone (abscissa) in GR 205171- and vehicle-treated mice. *p < 0.05, as compared with the respective inhibition in control mice (Student’s $t$-test).

**Effects of chronic administration of GR 205171 on [5-HT]ext in freely moving mice**

**Basal outflow in the frontal cortex and the DRN**

Under our microdialysis conditions, [5-HT]ext was relatively stable for the whole duration of experiments (180 min) allowing calculations of mean values in the frontal cortex and the DRN. The basal [5-HT]ext levels in these two brain regions were measured $\approx 20$ h after the removal of the osmotic mini pumps. The mean basal [5-HT]ext levels in the frontal cortex across all treatment groups were not
Table 1  Effects of chronic treatment with GR 205171 (10 mg/kg/d, 21 days) or its vehicle on the characteristics of 5-CT-evoked [35S]GTP-γ-S binding onto the DRN in brain sections

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>GR 205171 (10 mg/kg/d)</th>
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<tbody>
<tr>
<td>EC50 (nm)</td>
<td>13.6 ± 1.2 (4)</td>
<td>47.4 ± 16.1 (5)**</td>
</tr>
<tr>
<td>Emax (%)</td>
<td>137.4 ± 9.2 (4)</td>
<td>101.3 ±  5.1 (5)*</td>
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In vitro binding experiments and quantitative autoradiographic determinations were as described in the legend to Fig. 4. EC50 is the concentration of 5-CT inducing half-maximal [35S]GTP-γ-S binding, and Emax is the maximal 5-CT-evoked increase expressed as percentage over basal [35S]GTP-γ-S binding. Results are the means ± SEM of independent determinations in the number of mice indicated in parentheses. *p < 0.05, **p < 0.01, as compared with vehicle-treated mice (unpaired two-tailed Student’s t-test).

Effect of chronic treatment with GR 205171 (10 mg/kg/d, 21 days) or its vehicle on the characteristics of 5-CT-evoked [35S]GTP-γ-S binding onto the DRN in brain sections. Results are the means ± SEM of independent determinations in the number of mice indicated in parentheses. *p < 0.05, **p < 0.01, as compared with vehicle-treated mice (unpaired two-tailed Student’s t-test).
Injection of the selective 5-HT$_{1A}$ receptor antagonist, WAY 100635 (0.5 mg/kg, s.c.), 15 min prior to the paroxetine challenge, potentiated the increasing effect of the latter drug on cortical [5-HT]$_{\text{ext}}$ in control mice that received the vehicle for 21 days, but not in GR 205171-treated mice (Fig. 6a). Statistical analysis showed that cortical AUC values in control mice treated acutely with WAY 100635 and paroxetine reached those obtained in GR 205171-treated mice (184 ± 17 vs. 203 ± 14% (F$_{1,17}$ = 0.7; p = 0.4; Fig. 6b). The acute administration of WAY 100635 alone did not alter AUC values for the frontal cortex in these two groups of mice (data not shown).

**Discussion**

Because (i) 5-HT neurotransmission, and especially 5-HT$_{1A}$ autoreceptors, are critically involved in the central mechanisms of action of several classes of antidepressant drugs (Blier and De Montigny 1983; Le Poul et al. 2000), and (ii) NK$_1$ receptor antagonists are endowed with antidepressant properties (Kramer et al. 1998, 2004), our objective was to assess whether 5-HT$_{1A}$ autoreceptors might also be involved in the central effects of this novel class of potential antidepressants.

Our first series of in vitro electrophysiological experiments clearly showed that the selective NK$_1$ receptor antagonist GR 205171 (Zocchi et al. 2003) exerted no direct influence on DRN 5-HT neuron activity in mice, in line with previous data obtained with another NK$_1$ receptor antagonist, L-760735, in guinea pigs (Conley et al. 2002). Together with immunocytochemical observations which demonstrated that NK$_1$ receptors are not expressed by DRN 5-HT cells in mice (Froger et al. 2001), these electrophysiological results strongly suggest that, if it...
exists, substance P regulation of 5-HT neuronal activity is one of the most potent, selective (Gardner et al. 2003) or the vehicle (D) antagonists. However, acute injection of the effective dose of 10 mg/kg s.c. of GR 205171 (Rupniak et al. 2003; Hutson et al. 2004) affected neither the basal firing rate nor the potency of the 5-HT1A autoreceptor agonist, ipsapirone, to inhibit 5-HT neuron discharge in the DRN. In contrast, previous in vivo studies showed that acute administration of NK1 receptor antagonists caused a delayed increase in DRN neuronal firing rate (Santarelli et al. 2001; Conley et al. 2002). However, investigations in rats led to the conclusion that such a treatment did not modify the 5-HT1A autoreceptor-mediated inhibitory effect of 8-OH-DPAT on DRN 5-HT neuron firing in vivo (Haddjeri and Blier 2000). All these data support the idea that acute NK1 receptor blockade does not directly affect 5-HT1A autoreceptor functioning. However, under in vivo conditions, indirect network-dependent mechanisms (which are obviously lost in brainstem slices) might account for the delayed activation of DRN neurons (Santarelli et al. 2001; Conley et al. 2002).

In contrast, in mice that had been chronically treated with GR 205171, we found that the potency of ipsapirone to inhibit 5-HT neuron discharge in brainstem slices was clearly reduced, as expected from the occurrence of a functional desensitization of DRN 5-HT1A autoreceptors. This observation agrees with previous data obtained in NK1 receptor knock-out (NK1−/−) mice (Froger et al. 2001), although the amplitude of the adaptive change noted here after chronic blockade of NK1 receptors was less than that reported after genetic deletion of NK1 receptors. Previous studies in anaesthetized rats also showed such a desensitization of DRN 5-HT1A autoreceptors, associated with an increase in basal firing rate of 5-HT neurons, after short (2 days) and long (14 days) term treatments with another NK1 receptor antagonist, CP-96 345 (Haddjeri and Blier 2001). However, in guinea pigs, a 28-day treatment with L-760735, another potent NK1 receptor antagonist, was found to increase in vivo basal firing rate of 5-HT neurons without modifying the functional characteristics of DRN 5-HT1A autoreceptors (Conley et al. 2002). These discrepancies might be explained, at least in part, by well-established species differences (rat and mouse vs. guinea pig) in the pharmacological properties of NK1 receptors (see Conley et al. 2002).

In order to probe further 5-HT1A autoreceptors, we measured 5-HT1A autoreceptor-mediated binding of [35S]GTP-γ-S onto the DRN in brainstem sections. In both vehicle- and GR 205171-treated mice, the 5-HT1 receptor agonist 5-CT produced a concentration-dependent increase in [35S]GTP-γ-S autoradiographic labelling, which could be completely prevented by WAY 100635, as expected from its mediation through 5-HT1A autoreceptor activation (see also Fabre et al. 2000; Pejchal et al. 2002). Clearly, chronic administration of GR 205171 induced a shift to the right of the 5-CT concentration–response curve, with a significant decrease in the maximal stimulation of [35S]GTP-γ-S binding, which could indicate that the number of G proteins to be recruited by 5-HT1A receptor activation was reduced in GR 205171-treated compared with vehicle-treated mice. This alteration is similar to that previously found in NK1−/− mutants, where a reduction in the maximal 5-CT-evoked stimulation of [35S]GTP-γ-S binding onto the DRN was observed in comparison with NK1+/+ wild-type mice (Froger...
et al. 2001). Interestingly, it also corresponds to the adaptive change in 5-HT\textsubscript{1A} autoreceptor-mediated [\textsuperscript{35}S]GTP-$\gamma$-S binding already noted after 5-HT transporter (5-HTT) inactivation, either by pharmacological blockade with SSRIs or by gene deletion (Fabre et al. 2000).

Because 5-HT\textsubscript{1A} autoreceptors mediate a negative feedback control on both synthesis and release of 5-HT (Hamon 1997), their desensitization occurring after chronic NK\textsubscript{1} receptor blockade may lead to an increased serotonergic neurotransmission, such as that found after chronic SSRI treatment (Bel and Artigas 1993) and in 5-HTT\textsuperscript{−/−} knockout mice (Fabre et al. 2000). We directly assessed this inference using in vivo intracebral microdialysis in freely moving animals. Indeed, we found no evidence for changes in basal cortical [5-HT]$\text{ext}$ in mice after a 3-week treatment with GR 205171. Similarly, no differences in cortical 5-HT outflow were previously noted between NK\textsubscript{1}−/− and wild-type mice (Froger et al. 2001). However, chronic GR 205171 treatment was found to almost double the basal [5-HT]$\text{ext}$ levels in the DRN and to potentiate paroxetine-induced increase in [5-HT]$\text{ext}$ levels in both the frontal cortex and the DRN.

To date, microdialysis studies have yielded rather inconsistent results regarding the desensitization of somatodendritic 5-HT\textsubscript{1A} autoreceptors following chronic treatment with SSRIs. Several studies demonstrated increases in cortical [5-HT]$\text{ext}$ levels after sustained treatments with these drugs (Bel and Artigas 1993; Invernizzi et al. 1994; Hervas et al. 2001; Dawson et al. 2002), but we found no changes in basal cortical 5-HT outflow following chronic paroxetine treatment in rats (Malagie et al. 2000) and in mice (Gardier et al. 2003), such as that found here after a long-term treatment with GR 205171. Although no data are available in mice, SSRI treatments have been shown to either increase (Malagie et al. 2000) or leave unchanged (Bel and Artigas 1993) [5-HT]$\text{ext}$ levels in the rat DRN. However, in the latter study, microdialysis experiments were performed when osmotic mini pumps were still in place (Bel and Artigas 1993). Here, in contrast, as no pharmacokinetic data concerning GR 205171 have yet been reported, pumps were removed $\approx$20 h before performance of our microdialysis experiments in order to manage a significant washout period.

Interestingly, the selective 5-HT\textsubscript{1A} receptor antagonist WAY 100635 (Fletcher et al. 1996), failed to alter paroxetine-induced increase in cortical [5-HT]$\text{ext}$ levels in GR 205171-treated mice, further suggesting that 5-HT\textsubscript{1A} autoreceptors were desensitized in the latter animals as in SSRI-treated rodents (see Le Poul et al. 2000).

Because our electrophysiological data suggest that NK\textsubscript{1} receptor-mediated regulation of 5-HT neuron activity is indirect, it can be inferred that the changes in 5-HT neurotransmission observed after long-term blockade of NK\textsubscript{1} receptors probably involved complex neuronal networks. Interestingly, Chen et al. (2000) provided evidence that NK\textsubscript{1} receptors are expressed by noradrenergic neurons in the locus coeruleus, thus raising the possibility that substance P regulates the activity of these neurons and, indirectly, that of 5-HT neurons. In contrast, the lateral habenula and the amygdala, two regions which have close anatomical-functional relationships with DRN 5-HT neurons and are endowed with NK\textsubscript{1} receptors (Quartara and Maggi 1997; Peyron et al. 1998; Levita et al. 2003), might also be involved in NK\textsubscript{1} receptor antagonist-induced modulations of 5-HT neurotransmission (see Conley et al. 2002). Finally, local acting NK\textsubscript{1} receptor antagonists within the DRN should also be considered. Indeed, Liu et al. (2002) found that substance P acts primarily on local glutamatergic neuronal afferents to produce excitatory postsynaptic currents in 5-HT neurons. In addition, NK\textsubscript{1} receptors have been clearly identified on GABAergic neurons surrounding 5-HT cell bodies in the DRN (Ma and Bleasdale 2002), and their blockade might also contribute to enhanced 5-HT neurotransmission in mice chronically treated with GR 205171.

All these hypotheses should be addressed in order to elucidate the mechanisms responsible for 5-HT\textsubscript{1A} autoreceptor desensitization which probably contributes to the possible antidepressant action of chronic NK\textsubscript{1} receptor blockade, like that evidenced for SSRI antidepressants.

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