Blockade of substance P (neurokinin 1) receptors enhances extracellular serotonin when combined with a selective serotonin reuptake inhibitor: an in vivo microdialysis study in mice

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Abstract
Substance P antagonists of the neurokinin-1 receptor type (NK1) are gaining growing interest as new antidepressant therapies. It has been postulated that these drugs exert this putative therapeutic effect without direct interactions with serotonin (5-HT) neurones. Our recent microdialysis experiment performed in NK1 receptor knockout mice suggested evidence of changes in 5-HT neuronal function (Froger et al. 2001). The aim of the present study was to evaluate the effects of coadministration of the selective 5-HT reuptake inhibitor (SSRI) paroxetine with a NK1 receptor antagonist (GR205171 or L733060), given either intraperitoneally (i.p.) or locally into the dorsal raphe nucleus, on extracellular levels of 5-HT ([5-HT]ext) in the frontal cortex and the dorsal raphe nucleus using in vivo microdialysis in awake, freely moving mice. The systemic or intraraphe administration of a NK1 receptor antagonist did not change basal cortical [5-HT]ext in mice. A single systemic dose of paroxetine (4 mg/kg; i.p.) resulted in a statistically significant increase in [5-HT]ext with a larger extent in the dorsal raphe nucleus (+ 138% over basal AUC values), than in the frontal cortex (+ 52% over basal AUC values). Co-administration of paroxetine (4 mg/kg; i.p.) with the NK1 receptor antagonists, GR205171 (30 mg/kg; i.p.) or L733060 (40 mg/kg; i.p.), potentiated the effects of paroxetine on cortical [5-HT]ext in wild-type mice, whereas GR205171 (30 mg/kg; i.p.) had no effect on paroxetine-induced increase in cortical [5-HT]ext in NK1 receptor knock-out mice. When GR205171 (300 μmol/L) was perfused by ‘reverse microdialysis’ into the dorsal raphe nucleus, it potentiated the effects of paroxetine on cortical [5-HT]ext, and inhibited paroxetine-induced increase in [5-HT]ext in the dorsal raphe nucleus. Finally, in mice whose 5-HT transporters were first blocked by a local perfusion of 1 μmol/L of citalopram into the frontal cortex, a single dose of paroxetine (4 mg/kg i.p.) decreased cortical 5-HT release, and GR205171 (30 mg/kg i.p.) reversed this effect. The present findings suggest that NK1 receptor antagonists, when combined with a SSRI, augment 5-HT release by modulating substance P/5–HT interactions in the dorsal raphe nucleus.

Keywords: depression, frontal cortex, intracerebral microdialysis, NK1 receptor antagonist, selective serotonin reuptake inhibitor, substance P.


The clinical efficacy of antidepressant drugs such as selective serotonin re-uptake inhibitors (SSRIs) is based on facilitation of central serotonergic neurotransmission subsequent to the selective blockade of the serotonin (5-hydroxytryptamine, 5-HT) transporter. SSRIs are attractive drugs because they produce fewer adverse-effects than the first class of antidepressants, the tricyclics. However, SSRIs have in common with all other antidepressant drugs several drawbacks such as the length of delay to achieve clinical benefits in depressed patients. Received April 9, 2003; revised manuscript received November 19, 2003; accepted November 24, 2003.

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Abbreviations used: AUC, area under the curve; 5-HT, serotonin; NK, neurokinin; NA, noradrenaline; SSRI, selective serotonin reuptake inhibitor.
patients and that ≈30% of patients are resistant to the treatment. These limitations led to consideration of alternative strategies to improve their therapeutic efficacy.

One of the latest developments in the field of antidepressant drug therapy is an increased interest in neuropeptides such as substance P, which belongs to the family of tachykinins with two others neuropeptides, neurokinins A and B. Three types of tachykinin receptors, neurokinin-1 (NK1), NK2 and NK3 exhibiting preferences for substance P, neurokinin A and neurokinin B, respectively, have been identified (Regoli et al. 1994). The tachykinins have the peculiarity of being not highly selective for any given receptor type. Indeed, substance P binds and activates the G-protein coupled NK1 receptor, but also NK2 and NK3 receptors with a lower affinity (Maggi et al. 1993). In rodents, NK1 receptors have been identified in the hypothalamus (Liu et al. 2002), in the limbic structures (Beaujouan et al. 2000), in the locus coeruleus (Chen et al. 2000) and the dorsal raphe nucleus (Froger et al. 2001; Commons and Valentino 2002). It has been reported that the NK1 receptor antagonist MK-869 displayed antidepressant effects in a placebo-controlled trial performed in patients with a major depressive disorder (Kramer et al. 1998; Ranga and Krishnan 2002). Thus, these clinical data suggest that NK1 receptor antagonists may represent a putative new pharmacological class of antidepressant drugs.

The precise central effects of NK1 receptor antagonists now need to be clarified, as it has been claimed that these latter drugs do not act directly on monoamine systems like all other major types of antidepressant treatments already available for humans (Kramer et al. 1998; Rupniak et al. 2001). This assertion seems to be surprising since central interactions between substance P and serotonergic system have already been suggested in preclinical studies (Walker et al. 1991; Compan et al. 1997). It is thus tempting to presume that NK1 receptor antagonists could interact indirectly with neuronal 5-HT and/or noradrenergic systems in the brain (Haddjeri and Blier 2000).

We recently investigated this hypothesis using several approaches. For example, using in vivo microdialysis, we found evidence for alterations in 5-HT neuronal function in the frontal cortex of NK1 receptor knockout mice (Froger et al. 2001). The increase in the extracellular levels of 5-HT ([5-HT]ext) in the frontal cortex caused by a systemic administration of the SSRI, paroxetine, was four- to sixfold higher in freely moving NK1 receptor knock-out mice than in wild-types (Froger et al. 2001), suggesting the existence of critical interactions between 5-HT and substance P in the brain. In an attempt to find the brain region in which these interactions may occur, double immunocytochemical labelling experiments were performed by Moratalla’s group (Madrid, Spain) and others. No colocalization of 5-HT and NK1 receptors in the dorsal raphe nucleus cell bodies of wild-type mice was reported (Froger et al. 2001; Santarelli et al. 2001). Our knowledge of the central effects of NK1 receptor antagonists is further limited by some negative results obtained following administration of a single dose of NK1 receptor antagonists to rodents. Low doses of these compounds did not modify by themselves the firing activity of 5-HT neurones located in the dorsal raphe nucleus in rats (Haddjeri and Blier 2000), although acute higher doses or sustained pharmacological blockade of NK1 receptors increased spontaneous firing activity of the dorsal raphe nucleus 5-HT neurones (Haddjeri and Blier 2001; Conley et al. 2002). These latter results disagree with an intracerebral in vivo microdialysis study suggesting that NK1 receptor antagonists did not modify basal [5-HT]ext in the rat prefrontal cortex (Millan et al. 2001).

In this study, we show that the substance P system play a key role in the modulation of the brain serotonergic neurotransmission, since a single dose of a NK1 receptor antagonist [given either intraperitoneally (i.p.) or perfused locally into the dorsal raphe nucleus] potentiated the paroxetine response on dialysate 5-HT levels in the frontal cortex of mice.

Materials and methods

Animals
Male C57BL/6 wild-type and substance P (neurokinin-1) receptor knockout mice, 5–6 weeks old, weighing 25–35 g, were used in this study. Mutant mice derived from heterozygote crossings NK1 +/− couples raised on 129/Sv × C57BL/6 genetic background were made at the animal facility of University College, London (De Felice et al. 1998). The founders were shipped to France and their offspring were bred and reared on a C57BL/6 genetic background as heterozygote colonies and genotyped by PCR as already described (Froger et al. 2001). All animals were housed in animal care facility in groups of 3–6 and kept under standard conditions (room temperature of 22–23°C, 12 h : 12 h light–dark cycle, 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 87–848, 19 October 1987, Ministère de l’Agirculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 005307 to A.M. Gardier).

Drugs and treatment
GR205171 was a generous gift from GlaxoSmithKline laboratory (Marly le-roi, France). L733060 and L733061 were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). The selective 5-HT reuptake inhibitors, paroxetine hydrochloride was a generous gift from GlaxoSmithKline (Harlow, UK) and citalopram from Lundbeck laboratory (Copenhagen, Denmark). All chemical compounds such as GR205171, L733060, L733061, paroxetine and citalopram were dissolved in distilled water. Paroxetine was administered at the dose of 4 mg/kg and the NK1 receptor antagonists at the dose of 10, 30 or 40 mg/kg by the i.p. route. Control animals were injected using the appropriate vehicle and the
same administration route. For their local administration into the
dorsal raphe nucleus or the frontal cortex, GR205171 (30 or
300 μmol/L) and citalopram (1 μmol/L) were dissolved in artificial
cerebrospinal fluid (CSF) and perfused at a flow rate of 1.5 μL/min
and 0.5 μL/min, respectively.

Microdialysis procedure
Concentric dialysis probes were made of cuprophane fibres and set
up as described previously (Malagie et al. 1996). All probes
presented an active length of 2 and 1 mm for the frontal cortex
and the dorsal raphe nucleus, respectively (× 0.30 mm outer
diameter). Animals were anaesthetized with chloral hydrate
(400 mg/kg, i.p.). They were placed in a stereotaxic frame and
were implanted with a probe according to the ‘Mouse brains’ atlas
(Hof et al. 2000): coordinates from Bregma (in mm), the frontal
cortex: anterior = 1.6, lateral = 1.3, ventral = −1.6; the dorsal
raphe nucleus: anterior = −4.5, lateral = 0, ventral = −3.
Animals were allowed to recover from surgery overnight. The next
day, ≈20 h after the surgery, the probe was continuously perfused
with artificial CSF (composition in mmol/l: NaCl 147, KCl 3.5,
CaCl₂ 1.26, MgCl₂ 1.2, NaH₂PO₄ 1.0, pH 7.4 ± 0.2) at a flow rate
of 1.5 μL/min in the frontal cortex and 0.5 μL/min in the dorsal
raphe nucleus using CMA/100 pump (Carnegie Medicin, Stock-
holm, Sweden). Dialysates were collected every 15 min for the
frontal cortex and every 30 min for the dorsal raphe nucleus in a
small Eppendorf tube for the measurements of their 5-HT contents
using a high performance liquid chromatography (HPLC) system
(XL-ODS, 4.6 x 75 mm, particle size 3 μm; Beckman) coupled to
an amperometric detector (1049 A, Hewlett-Packard, Les Ulis,
France). Four fractions were collected to measure basal values
(mean ± SEM) before systemic administration of the drugs. The
limit of sensitivity for [5-HT]ext was ≈0.5 fmol per sample
(signal-to-noise ratio = 2). At the end of the experiments, the
placement of microdialysis probes was verified histologically.

Histological verification of microdialysis probes implantation
After the microdialysis study, mice were killed by cervical
dislocation, brains were removed and stored at −40°C. To determine
the exact probe implantation in the frontal cortex or dorsal raphe
nucleus, brains were placed in a Kryomat apparatus and kept at
−25°C. Brain regions were identified according to Hof et al. (2000)
‘Mouse brains’ atlas and coronal frozen section of brain sliced
serially at 40-μm intervals. Slices were realized from AP 1 to 2 mm
and AP = 4 to −5 mm for the frontal cortex and the dorsal raphe
nucleus, respectively. Each slice was photographed using a digital
camera (PowerShot G1, Canon) and the implantation of the probe
estimated in comparison to the corresponding slices obtained by the
‘Mouse brains’ atlas software (Hof et al. 2000). Only mice with
probes confined to either the frontal cortex or the dorsal raphe
nucleus were used for subsequent data analysis (examples of a probe
implantation in the frontal cortex and the dorsal raphe nucleus are
given in Fig. 6a,b, respectively).

Experimental protocols
In this study, all experiments were performed to determine the
effects of substance P (neurokinin-1) receptor antagonists on
extracellular 5-HT levels ([5-HT]ext) in the frontal cortex and the
dorsal raphe nucleus of mice.

Experiment 1. Effect of NK1 receptor antagonists administered i.p.
on cortical [5-HT]ext of wild-type mice. Following collection of
four baseline dialysate samples, freely moving wild-type mice were
administered with either the vehicle or various NK1 receptor
antagonists, GR205171 (30 mg/kg; i.p.) or L733060 (40 mg/kg;
i.p.). Dialysate samples were collected for a 0–120 min post-
treatment period.

Experiment 2. Effect of NK1 receptor antagonists administered i.p.
on cortical [5-HT]ext of paroxetine-treated wild-type mice. Follow-
collection of four baseline dialysate fractions, freely moving
wild-type mice were administered first with paroxetine (4 mg/kg;
i.p.), then 1 h later, with either vehicle, GR205171 (10 and 30 mg/kg;
i.p.), L733060 (active enantiomer) or L733061 (low-affinity
enantiomer) (40 mg/kg; i.p.) and dialysates were collected for an
additional 1 h.

Experiment 3. Effect of NK1 receptor antagonist administered i.p.
on cortical [5-HT]ext of paroxetine-treated NK1 receptor knockout
mice. Following collection of four baseline dialysate fractions,
freely moving NK1 receptor knock-out mice received first paroxe-
tine (4 mg/kg; i.p.), then 1 h later, either vehicle or GR205171
(30 mg/kg; i.p.) and dialysate samples were collected for an
additional 1 h.

Experiment 4. Effect of intraraphe perfusion of NK1 receptor
antagonist on [5-HT]ext levels in the dorsal raphe nucleus of
paroxetine-treated wild-type mice. Following collection of four
baseline dialysate fractions, freely moving wild-type mice received
first vehicle or paroxetine (4 mg/kg; i.p.), then 1 h later, a
continuous perfusion of vehicle or GR205171 (30 or 300 μmol/L)
locally into the dorsal raphe nucleus for 1 h, and dialysate samples
were collected for this additional 1 h.

Experiment 5. Effect of intraraphe perfusion of selective NK1
receptor antagonist on cortical [5-HT]ext of paroxetine-treated wild-
type mice. Following collection of four baseline dialysate fractions,
freely moving wild-type mice received first vehicle or paroxetine
(4 mg/kg; i.p.), then 1 h later, a continuous perfusion of vehicle or
GR205171 (30 or 300 μmol/L) locally into the dorsal raphe nucleus
for 1 h, and dialysates samples were collected for this additional 1 h.

Experiment 6. Effect of a NK1 receptor antagonist administered i.p.
on cortical 5-HT release of wild-type mice. The 5-HT transporter
was blocked in the frontal cortex by a local continuous perfusion of
citalopram (1 μmol/L). Following collection of four baseline
dialysate fractions, freely moving wild-type mice received first
vehicle or paroxetine (4 mg/kg; i.p.), then 1 h later, either vehicle or
GR205171 (30 mg/kg; i.p.), and dialysate samples were collected
for an additional 1 h.

Data analysis and statistics
The basal value of [5-HT]ext was calculated from the mean of the
four first samples collected. All subsequent sample values calculated
as the amount of 5-HT outflow collected during the 0–120 min
period from the frontal cortex are expressed as a percentage of basal
values. Statistical analyses, using the computer software StatView
Results

Effects of systemic injection of the NK1 receptor antagonists GR205171 or L733060 on extracellular 5-HT levels in the frontal cortex of wild-type mice

Basal cortical extracellular levels of 5-HT in groups of mice treated with either vehicle, GR205171 (30 mg/kg; i.p.) or L733060 (40 mg/kg; i.p.) were (in fmol/20 μL) 3.38 ± 0.48 (n = 5); 3.50 ± 0.92 (n = 5) and 4.26 ± 0.61 (n = 5), respectively, and did not significantly differ between the three groups of mice [F(2,12) = 1.84, p = 0.19]. Neither the vehicle nor NK1 receptor antagonists such as GR205171 (30 mg/kg) or L733060 (40 mg/kg) administered intraperitoneally modified the basal cortical [5-HT]ext during the 0–120 min post-treatment period [F(2,12) = 0.37, p = 0.69] (Fig. 1a,b).

Effects of systemic injection of the NK1 receptor antagonists GR205171, L733060 or L733061 on extracellular 5-HT levels in the frontal cortex of paroxetine-treated wild-type mice

Basal cortical extracellular levels of 5-HT in mice treated with either paroxetine/vehicle, paroxetine/GR205171 (10 and 30 mg/kg; i.p.), paroxetine/L733060 (40 mg/kg; i.p.) or paroxetine/L733061 (40 mg/kg; i.p.) were (in fmol/20 μL) 2.85 ± 0.35 (n = 9); 4.66 ± 0.34 (n = 5); 3.18 ± 0.25 (n = 8); 3.01 ± 0.17 (n = 7) and 3.63 ± 0.39 (n = 6), respectively. These values did not significantly differ between these various paroxetine-treated groups [F(4,30) = 1.24, p = 0.31].

In the frontal cortex, the paroxetine/vehicle treatment, as compared with a vehicle/vehicle treatment, increased significantly the [5-HT]ext during the t0–t60 period (AUC values were: 159 ± 20% versus 90 ± 8%, respectively) [F(1,12) = 7.18, p = 0.02], but only marginally during the t60–t120 period (AUC values were: 135 ± 16% versus 99 ± 10%, respectively) [F(1,12) = 2.08, p = 0.17: not statistically significant]. Before the intraperitoneal administration of NK1 receptor antagonists (from t0 to t60), increases in cortical [5-HT]ext were not significantly different in the various paroxetine-treated groups [F(4,41) = 1.53, p = 0.22].

Then, Analysis of AUC values, calculated for the amount of 5-HT collected in the frontal cortex during the 60–120 min post-NK1 receptor antagonists treatment, were significantly higher in groups which received a single dose of paroxetine (4 mg/kg; i.p.) and GR205171 (30 mg/kg; i.p.; §p < 0.05) or L733060 (40 mg/kg; i.p.; §p < 0.05) than the AUC value of the paroxetine (4 mg/kg; i.p.)/vehicle-treated group [F(3,35) = 5.41, p = 0.001] (Fig. 2b,d, respectively). The AUC value in the group of mice receiving paroxetine and the low-affinity enantiomer L733061 was not statistically different as compared with the paroxetine/vehicle-treated group (p = 0.49).

Thus, the NK1 receptor antagonists GR205171 and L733060 enhanced cortical extracellular 5-HT levels after paroxetine injection in wild-type mice. This effect might be the consequence of either a reduced reuptake or an increased release of 5-HT in the frontal cortex.
Effect of systemic injection of the NK1 receptor antagonist GR205171 on extracellular 5-HT levels in the frontal cortex of paroxetine-treated NK1 receptor knockout mice

Basal cortical extracellular levels of 5-HT in knockout mice treated with either paroxetine/vehicle (○) or paroxetine/GR205171 (10 mg/kg; i.p.) were (in fmol/20 μL) 3.57 ± 0.40 (n = 7) and 3.28 ± 0.13 (n = 8), respectively. These values did not significantly differ between these two paroxetine-treated groups [F1,13 = 0.52, p = 0.48].

Before the intraperitoneal administration of either vehicle or GR205171 (30 mg/kg) (from t0 to t60), increases in [5-HT]ext were not significantly different in the various paroxetine-treated groups [F1,13 = 0.46, p = 0.50]. Then, analysis of AUC values calculated for the amount of 5-HT outflow measured during the 60–120 min post-treatment period and expressed as percentages of baseline (n = 9–12 mice per group). *p < 0.05; **p < 0.001 significantly different from vehicle/vehicle treated group; §p < 0.05; significantly different from paroxetine/vehicle treated group (one-way ANOVA followed by a PLSD post hoc test). Prx, paroxetine; i.p., intraperitoneal; ns, not statistically significant.

Effect of systemic injection of the NK1 receptor antagonist GR205171 on extracellular 5-HT levels in the frontal cortex of paroxetine-treated NK1 receptor knockout mice

Basal cortical extracellular levels of 5-HT in knockout mice treated with either paroxetine/vehicle or paroxetine/GR205171 (30 mg/kg; i.p.) were (in fmol/20 μL) 3.57 ± 0.40 (n = 7) and 3.28 ± 0.13 (n = 8), respectively. These values did not significantly differ between these two paroxetine-treated groups [F1,13 = 0.52, p = 0.48].

Before the intraperitoneal administration of either vehicle or GR205171 (30 mg/kg) (from t0 to t60), increases in [5-HT]ext were not significantly different in the various paroxetine-treated groups of mutant mice [F1,13 = 0.46, p = 0.50]. Then, analysis of AUC values calculated for the amount of 5-HT collected in the frontal cortex during the 60–120 min post-vehicle or GR205171 (30 mg/kg; i.p.) were not significantly different in these two groups of mutant mice receiving the paroxetine/vehicle and paroxetine/GR205171 (30 mg/kg; i.p.) treatments [F1,13 = 0.055, p = 0.81] (Fig. 3a time course study and Fig. 3b on AUC values).

Thus, the enhancing effect of GR205171 on cortical extracellular 5-HT levels after paroxetine injection in wild-type mice appears to be NK1 receptor specific.

Effects of intraraphe perfusion of the NK1 receptor antagonist GR205171 on extracellular 5-HT levels in the dorsal raphe nucleus of paroxetine-treated wild-type mice

Basal extracellular levels of 5-HT in the dorsal raphe nucleus of mice treated with either vehicle/vehicle, vehicle/GR205171 (300 μmol/L), paroxetine/vehicle or paroxetine/GR205171 (30 or 300 μmol/L) were (in fmol/10 μL) 9.97 ± 2.20 (n = 5); 5.84 ± 0.37 (n = 5); 7.90 ± 1.09 (n = 9); 6.13 ± 0.71 (n = 8) and 7.64 ± 0.80 (n = 10), respectively. These values did not significantly differ between these groups of mice [F4,32 = 1.32, p = 0.28].

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cell bodies located in the dorsal raphe nucleus following paroxetine injection.

**Effects of intraraphe perfusion of the NK1 receptor antagonist GR205171 on extracellular 5-HT levels in the frontal cortex of paroxetine-treated wild-type mice**

Basal cortical extracellular levels of 5-HT in mice treated with either vehicle/vehicle, vehicle/GR205171 (300 μmol/L), paroxetine/vehicle or paroxetine/GR205171 (30 or 300 μmol/L) were (in fmol/20 μL) 3.50 ± 0.61 (n = 5); 3.18 ± 0.34 (n = 8); 3.29 ± 0.57 (n = 6); 4.87 ± 0.52 (n = 6) and 5.49 ± 0.73 (n = 8), respectively. These values did not significantly differ between these groups of mice [F_{4,28} = 1.46, p = 0.075].

Before the intraraphe perfusion of GR205171 (30 and 300 μmol/L) (from t₀ to t₆₀), changes in [5-HT]ext in the frontal cortex were not significantly different in the various paroxetine-treated groups [F_{2,17} = 0.59, p = 0.94]. Then, the analysis of AUC values, calculated for the amount of 5-HT collected in the frontal cortex during the 60–120-min period of intraraphe GR205171 perfusion, revealed that the administration of a single dose of paroxetine (4 mg/kg; i.p.) and GR205171 300 μmol/L (but not 30 μmol/L), significantly potentiated the effect of paroxetine on cortical [5-HT]ext as compared with the AUC value of paroxetine/vehicle-treated group [F_{4,28} = 3.16, p = 0.03] (Fig. 4c,d). When given alone, intraraphe perfusion of GR205171 (300 μmol/L) did not modify basal cortical [5-HT]ext in mice.

Thus, the enhancing effect of GR205171 on cortical extracellular 5-HT levels after paroxetine injection appears to be mediated by brainstem cells.

**Effects of systemic injection of the NK1 receptor antagonist GR205171 on 5-HT release in the frontal cortex of paroxetine-treated wild-type mice**

In the presence of a local perfusion of citalopram (1 μmol/L) by ‘reverse microdialysis’ into the frontal cortex, basal cortical extracellular level of 5-HT in mice treated with either vehicle/vehicle, vehicle/GR205171 (30 mg/kg; i.p.), paroxetine/vehicle or paroxetine/GR205171 (30 mg/kg; i.p.) were (in fmol/20 μL) 12.62 ± 1.51 (n = 13); 12.13 ± 2.16 (n = 12); 10.75 ± 1.2 (n = 11) and 9.45 ± 0.57 (n = 11), respectively. These values did not significantly differ between these groups of mice [F_{3,42} = 0.92, p = 0.43].

In the presence of 1 μmol/L of citalopram, acute administration of paroxetine (4 mg/kg; i.p.)/vehicle decreased dialysate 5-HT in the frontal cortex over the 0–120 min of dialysate collection (Fig. 5). Before administration of either the vehicle or GR205171 (from t₀ to t₆₀), decreases in cortical [5-HT]ext were not significantly different in both paroxetine-treated groups [F_{1,20} = 0.60, p = 0.44]. Administration of a single dose of GR205171 (30 mg/kg; i.p.) 1 h after paroxetine, reduced paroxetine-induced decrease in cortical 5-HT levels (from t₇₅ to t₁₂₀) [F_{7,145} = 7.83, p < 0.001]. Differences
between the two groups were statistically significant at $t_{90}$ ($p < 0.01$), $t_{105}$ ($p < 0.001$) and $t_{120}$ ($p < 0.001$) (Fig. 5). In the presence of a local perfusion of citalopram in the frontal cortex, GR205171 alone (30 mg/kg; i.p.) did not modify cortical [5-HT]ext as compared with the group of wild-type mice treated with vehicle only ($p = 0.79$) (Fig. 5).

Thus, the enhancing effect of GR205171 on cortical extracellular 5-HT levels after paroxetine injection could be attributable to an increase in 5-HT release, at least partly.

**Discussion**

In the present study, using in vivo microdialysis in awake, freely moving mice, we found that the effects of the SSRI, paroxetine, on cortical [5-HT]ext, were potentiated by a systemic administration of the NK1 receptor antagonists, GR205171 and L733060. These effects were not observed in NK1-R KO mice. In addition, the local perfusion of GR205171 through the dialysis probe implanted into the dorsal raphe nucleus in wild-type mice reproduced the effects of its systemic administration at serotonergic nerve terminals, while opposite changes were found in the dorsal raphe nucleus with a decrease in extracellular 5-HT levels.

In a first experiment, we evaluated the effects of the NK1 receptor antagonists GR205171 and L733060 given alone on cortical [5-HT]ext in C57BL/6 wild-type mice. Consistent with results already obtained using in vivo microdialysis in the frontal cortex of rats (Millan et al. 2001) or CD-1 mice.
corresponding time (one-way significantly different from the paroxetine/vehicle-treated group at the predefined **

and/or the difference of efficacy of NK1 receptor antagonists L733060 and L733061 presumably due to the elevated dose analysis failed to achieve a clear separation between

(40 mg/kg; i.p.) had no significant effect. However, statistical

[(5-HT)ext whereas its low-affinity enantiomer, L733061

significantly potentiated the effects of paroxetine on cortical

changes in cortical [5-HT]ext. L733060 (40 mg/kg; i.p.)

administration of NK1 receptor antagonists to rats (Zocchi e

et al. 2003), we found that the blockade of NK1 receptors did not affect basal cortical [5-HT]ext. These microdialysis data agree with the absence of modifications of the spontaneous firing activity of 5-HT neurones located in the dorsal raphe nucleus following a single, systemic administration of NK1 receptor antagonists to rats (WIN51708, CP96345: Haddjeri and Blier 2000; GR205171: Lejeune et al. 2002).

Then, we tested for the effects of a coadministration of paroxetine with either an active NK1 receptor antagonist (GR205171, L733060) or a low-affinity enantiomer (L733061) on cortical [5-HT]ext in mice. A systemic administration of a single dose of GR205171 (30 mg/kg; i.p.) significantly potentiated the effects of paroxetine on cortical [5-HT]ext. These results obtained in mice concur with studies reporting that GR205171 is a brain penetrant compound, exhibits a high affinity for the NK1 receptors in rats (Gardner et al. 1996; Rupniak et al. 2000) and displaces the binding of [3H]-SP with a similar efficacy in mice compared to rats (Zocchi et al. 2003). Based on the high affinity of L733060 for NK1 receptors and its good brain penetration in human and the guinea-pig (Kramer et al. 1998), we also evaluated its effects on paroxetine-induced changes in cortical [5-HT]ext. L733060 (40 mg/kg; i.p.) significantly potentiated the effects of paroxetine on cortical [5-HT]ext whereas its low-affinity enantiomer, L733061 (40 mg/kg; i.p.) had no significant effect. However, statistical analysis failed to achieve a clear separation between L733060 and L733061 presumably due to the elevated dose and/or the difference of efficacy of NK1 receptor antagonists between some species because L733060 was shown to produce enantioselective central effects in gerbils or guinea pigs at the dose of 10 mg/kg after its systemic administration (Rupniak et al. 1996; Rupniak et al. 2000).

Because these compounds have a lower affinity for the mouse NK1 receptors compared to human or guinea pig, we had to administer relatively high doses of NK1 receptor antagonists (30 or 40 mg/kg). Thus, unspecified effects could have occurred through the blockade of either NK2/NK3 receptors or calcium channels after their systemic administration (Rupniak et al. 1993, 1994). Here, a control study using NK1 receptor knockout mice, recently generated by De Felipe et al. (1998), assessed the selectivity of the cortical serotonergic effects of the systemic coadministration of paroxetine and GR205171. GR205171 administered i.p. did not alter the effects of paroxetine on cortical [5-HT]ext in these mutants. These data confirm that, in wild-type mice, the selective blockade of brain NK1 receptors, but not that of NK2 or NK3 receptors, by GR205171 and L733060, mediated the potentiation of the effects of paroxetine on cortical [5-HT]ext. It is of interest to consider these results in light of recent studies showing that GR205171 (30 mg/kg) displayed antidepressant-like activity (Zocchi et al. 2003) and inhibited neonatal vocalizations in mice with a marginal enantioselectivity (Rupniak et al. 2000; Rupniak et al. 2003). Taken together, our result suggest that activation of NK1 receptors by endogenous substance P in the dorsal raphe nucleus may limit the effects of a single dose of SSRI.
on extracellular 5-HT levels at serotonergic nerve terminals in mice. However, in this inhibitory effect on neuronal 5-HT activity through the activation of NK1 receptors, we cannot exclude the involvement of the endogenous neurokinin A whose mRNA was also identified in the dorsal raphe nucleus (Harlan et al. 1989).

Then, to specify one possible central site of action of the NK1 receptor antagonist, we perfused GR205171 locally into the dorsal raphe nucleus, the main source of brain serotonergic neurones. First, we found that a single dose of paroxetine-induced a higher increase in [5-HT]ext in the dorsal raphe nucleus (AUC values = +138% above baseline) than in the frontal cortex (AUC values = +52%). These results obtained in mice agree with those already described in rats following SSRI administration (Bel and Artigas 1992). When paroxetine was coadministered with GR205171 perfused into the dorsal raphe nucleus, the lower concentration used (30 μmol/L) did not change dialysate 5-HT in both the dorsal raphe nucleus and the frontal cortex. However, intraraphe perfusion of GR205171 at a 10 fold higher concentration (300 μmol/L), reduced the increase in raphe 5-HT induced by paroxetine and simultaneously potentiated the effect of paroxetine in the frontal cortex presumably through a less feedback inhibition of the serotonergic neurones (Artigas 1993).

Finally, several hypotheses can be considered regarding the mechanism by which NK1 receptor antagonists induced changes in cortical extracellular levels of 5-HT when combined with a SSRI. In vivo intracerebral microdialysis technique is recognized as a presynaptic test measuring a balance between release and reuptake of neurotransmitters such as 5-HT (Ungerstedt 1984). Thus, to discriminate between these two processes, 5-HT transporters in wild-type mice were first blocked by a continuous ‘reverse-dialysis’ perfusion of a 5-HT reuptake inhibitor (citalopram 1 μmol/L) into the 5-HT nerve terminal area, the frontal cortex (Hjorth and Auerbach 1994). Mice then received a systemic administration of paroxetine, which decreased cortical [5-HT]ext, because it blocked 5-HT reuptake in the dorsal raphe nucleus and, in turn, activated somatodendritic 5-HT₁A autoreceptors by endogenous 5-HT. The magnitude of this decrease was comparable to that previously described with the SSRI by endogenous 5-HT. The magnitude of this decrease was comparable to that previously described with the SSRI by endogenous 5-HT. The magnitude of this decrease was comparable to that previously described with the SSRI by endogenous 5-HT. The magnitude of this decrease was comparable to that previously described with the SSRI by endogenous 5-HT. It is noteworthy that the serotonergic nerve terminals.

ing paroxetine-induced decrease in 5-HT release at brain serotonergic pathway, however, whether somatodendritic 5-HT₁A autoreceptors play a key role in these interactions needs to be clarified.

References


