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ACCELERATED COMMUNICATION

Substance P Neurokinin 1 Receptor Activation within the Dorsal Raphe Nucleus Controls Serotonin Release in the Mouse Frontal Cortex

Bruno P. Guiard, Jean-Philippe Guilloux, Christelle Reperant, Stephen P. Hunt, Miklos Toth, and Alain M. Gardier

Faculté de Pharmacie, Université Paris-Sud, Châtenay-Malabry, France (B.P.G, J.P.G, C.R., A.M.G.); Department of Anatomy and Developmental Biology, University College London, London, United Kingdom (S.P.H.); and Department of Pharmacology, Weill Medical College of Cornell University, New York, New York (M.T.)

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ABSTRACT

Preclinical studies suggest that substance P (SP) neurokinin 1 (NK1) receptor antagonists are efficient in the treatment of anxiety and depression. This therapeutic activity could be mediated via stimulation of serotonin (5-HT) neurons located in the dorsal raphe nucleus (DRN), which receive important SP-NK1 receptor immunoreactive innervations. The present study examined the effects of intraraphe injection of SP on extracellular 5-HT levels in the frontal cortex, ventral hippocampus, and DRN by using intracerebral microdialysis in conscious mice. Intraraphe SP injection dose dependently decreased cortical 5-HT release, whereas no effects were detected in the ventral hippocampus. Cortical effects were blocked by the selective NK1 receptor antagonist *N*-[[2-methoxy-5-[5-(trifluoromethyl)tetrazol-1-yl]phenyl]methyl]-2-phenylpiperidin-3-amine (GR205171) and completely dampened in mice lacking NK1 receptors. Furthermore, genetic (in knockout 5-$HT_{1A}^{-/-}$ mice) or pharmacological inactivation of 5-HT_{1A} autoreceptors blocked cortical responses to SP. Contrasting with its cortical effects, intraraphe SP injection increased 5-HT outflow in the DRN in wild-type mice; this effect was potentiated by a local perfusion of the selective 5-HT_{1A} antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY100635). Finally, SP-induced changes in frontal cortex and DRN dialysate 5-HT levels were blocked by the DRN perfusion of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate ionotropic receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX). These data support the hypothesis that SP-induced overactivation of 5-HT_{1A} autoreceptors within the DRN limits cortical 5-HT release. A better knowledge of the complex relationship between tachykininergic, serotonergic, and glutamatergic systems within the DRN might help better understand the pathophysiology and subsequent treatment of depression.

Substance P (SP), a small peptide that belongs to the tachykinins family with neurokinins A and B, is widely distributed in the brain, specifically in limbic regions and brainstem nuclei such as the dorsal raphe nucleus (DRN) (Froger et al., 2001; Commons et al., 2002; Lacoste et al., 2006). In several species, including rodents and humans, SP distribution overlaps with that of its high-affinity NK1 receptor (Ribeiro-da-Silva and Hokfelt, 2000). Recent clinical and preclinical studies have pointed out the potential therapeutic action of SP (neurokinin 1) receptor antagonists in major depressive disorders (Kramer et al., 1998; Chahl, 2006). Data obtained from NK1 receptor knockout mice have suggested that the antidepressant-like action of NK1 receptor inactivation may result, at least in part, from an increase in central 5-HT neurotransmission through functional desensitization

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ABBREVIATIONS: SP, substance P; DRN, dorsal raphe nucleus; NK, neurokinin; 5-HT, serotonin; FC, frontal cortex; vH, ventral hippocampus; WAY100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-*N*-2-pyridinylcyclohexanecarboxamide; DNQX, 6,7-dinitroquinoxaline-2,3-dione; GR205171, *N*-[[2-methoxy-5-[5-(trifluoromethyl)tetrazol-1-yl]phenyl]methyl]-2-phenylpiperidin-3-amine; AUC, area under the curve; ANOVA, analysis of variance; i.r., intraraphe.

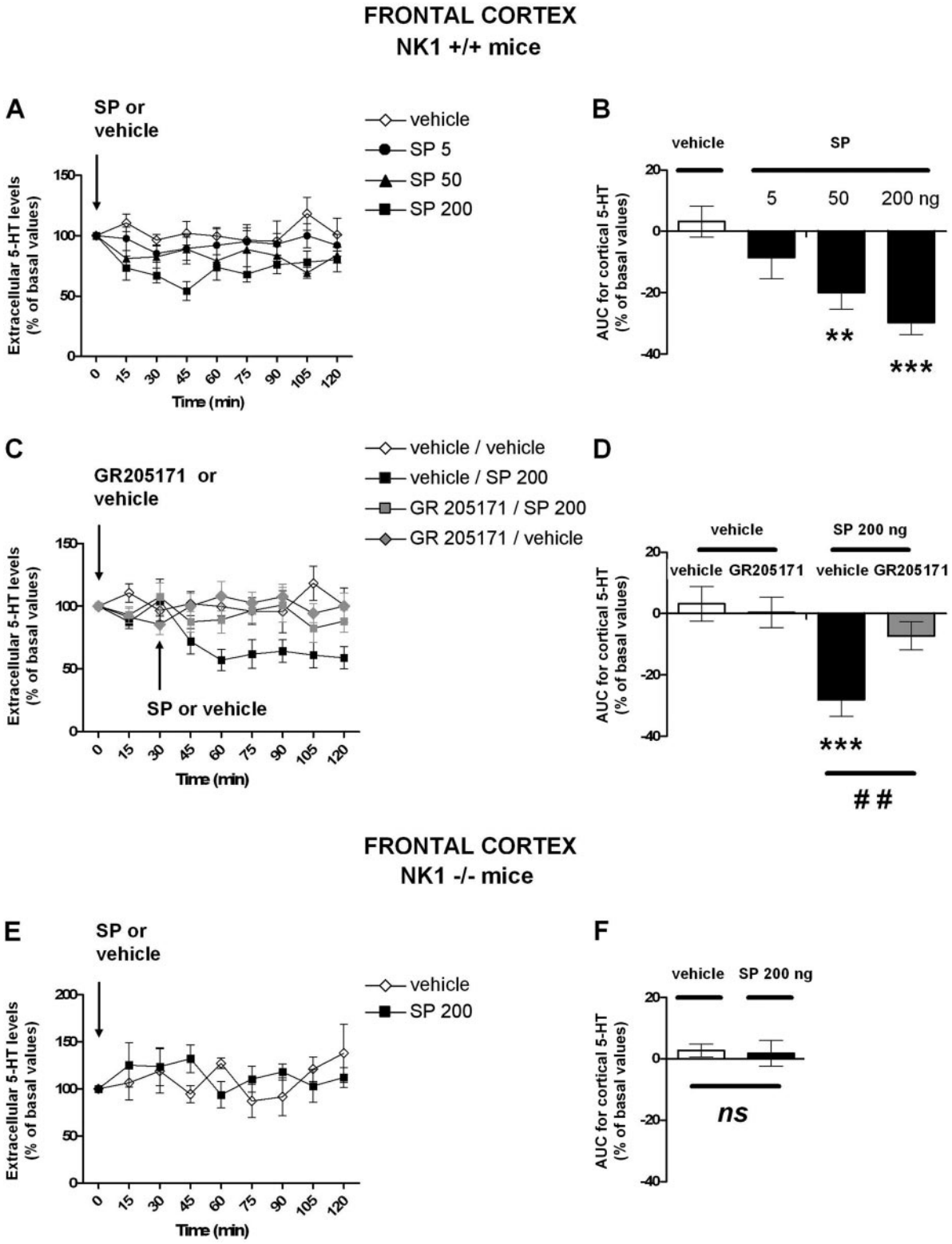

Fig. 1. Effect of intraraphe injection of substance P on 5-HT release in the frontal cortex mice. A, C, and E, data are means ± S.E.M. of extracellular 5-HT levels expressed as percentage of basal values (arrows show the time of drug injection). B, D, and F, data are AUC (means ± S.E.M.) values calculated for 5-HT outflow. B, one-way ANOVA on AUC values revealed a significant effect of treatment factor in the FC [$F_{(3,33)} = 6.1$; $p < 0.01$]. A pharmacological (D) and a genetic (F) inactivation of NK1 receptors were used to verify the selectivity of the SP response. D, two-way ANOVA on AUC values indicated a significant effect of pretreatment (vehicle or GR205171: 30 mg/kg; i.p.) [$F_{(1,31)} = 4.7$; $p < 0.05$], and treatment (vehicle or SP) [$F_{(1,31)} = 14.1$; $p < 0.001$] factors, but no significant interaction between these two factors [$F_{(1,31)} = 2.9$; $p > 0.05$] in the FC of $NK1^{+/+}$ mice. F, a Student's t test revealed no significant difference between the effect of vehicle and SP (200 ng) on extracellular 5-HT levels in the FC of $NK1^{-/-}$ mice. *ns*, not statistically significant. **, $P < 0.01$ and ***, $P < 0.001$ significantly different from vehicle-treated group. ##, $P < 0.01$, significantly different from vehicle/SP (200 ng)-treated group. The number of determinations (n) and means ± S.E.M. of baseline 5-HT levels expressed as femtomoles per sample for each experimental group in the FC were: vehicle ($n = 7$; 9.7 ± 0.7), SP 5 ($n = 11$; 7.9 ± 0.9), SP 50 ($n = 10$; 10.6 ± 0.8), and SP 200 ($n = 11$; 9.4 ± 0.6) (Fig. 1A); vehicle/vehicle ($n = 7$; 9.7 ± 0.7), GR205171/vehicle ($n = 11$; 10.1 ± 2.1), vehicle/SP 200 ($n = 11$; 8.6 ± 1.2), and GR205171/SP 200 ($n = 10$; 8.9 ± 0.5) (Fig. 1C); vehicle ($n = 7$; 10.4 ± 0.7), SP 200 ($n = 7$; 10.8 ± 1.2) (Fig. 1E). No significant differences were detected in baseline levels between experimental groups for individual experiments.

of 5-HT_{1A} autoreceptors located in the DRN (Froger et al., 2001). Pharmacological arguments from wild-type mice undergoing long-term treatment with an NK1 receptor antagonist also favor this hypothesis (Guiard et al., 2005). It is noteworthy that such a desensitization of somatodendritic 5-HT_{1A} autoreceptors resembles that induced by long-term treatment with selective serotonin reuptake inhibitors (Blier and de Montigny, 1980; Hjorth et al., 2000). Thus, the enhancement of serotonergic neurotransmission would be a common element in the antidepressant-like activity of both selective serotonin reuptake inhibitors and NK1 receptor antagonists.

Given evidence that NK1 receptor antagonists stimulate the DRN-5-HT system, it may be postulated that endogenous SP limits 5-HT release at serotonergic nerve terminals. However, initial in vitro electrophysiological recordings suggested that SP excites DRN 5-HT neurons via glutamatergic afferents (Liu et al., 2002). These findings were consistent with intracerebral in vivo microdialysis experiments indicating that SP injection into the DRN in conscious rats produces a small, transient increase in hippocampal 5-HT release (by >30% for 20 min compared with vehicle control) (Gradin et al., 1992). The latter findings have been challenged recently by in vivo electrophysiological data, suggesting that the effects of SP depended on the location of the recording within the DRN, with excitation predominating in the dorsal part of the DRN and inhibition more ventrally (Valentino et al., 2003). Thus, whether 5-HT neurotransmission were increased or decreased in projection brain regions of the DRN would depend on the specific DRN subregion that projects to the serotonergic nerve terminal area studied. Based on their findings, Valentino et al., (2003) have drawn an in vivo model of SP regulation of 5-HT neuronal activity. They propose that excitation of DRN 5-HT neurons synaptically linked with glutamate neurons expressing NK1 receptors allows 5-HT release and subsequent activation of somatodendritic 5-HT_{1A} autoreceptors. However, this theory is limited by the fact that 5-HT releasing properties of SP have yet to be demonstrated. We therefore employed both genetic and pharmacological approaches to clarify the interactions between SP and 5-HT_{1A} autoreceptors. To better define the effects of proximal and distal intraraphe SP injection on extracellular levels of 5-HT ([5-HT]ext), we performed intracerebral in vivo microdialysis studies in awake, freely moving mice with probes implanted either in a brain region containing numerous 5-HT nerve terminals (frontal cortex, ventral hippocampus) or in the vicinity of 5-HT cell bodies in the DRN.

Materials and Methods

Animals. Male C57BL/6 wild-type and NK1 receptor knock-out mice were derived from a stock of genotyped animals received from the animal facility of University College London (London, UK). As well, male wild-type and 5-HT_{1A} receptor knock-out mice also raised on a C57BL/6 genetic background were bred in our animal care facility (Univ. Paris XI, France). All animals were matched for age (8–10 weeks old) and weight (25–35 g) and were kept under standard housing conditions. Procedures involving animals and their care were conducted in conformity with the institutional guidelines, which are in compliance with national and international laws and policies (Council directive no 87-848, 19 October 1987, Ministere de l'Agriculture et de la Forêt, permission #92-196 to A.M.G.).

Microdialysis Procedure. Concentric dialysis probes were stereotaxically implanted under anesthesia (chloral hydrate, 400 mg/kg i.p.) into the frontal cortex (FC), ventral hippocampus (vH) (active length, 1.5 mm), or DRN (active length, 1.0 mm). Coordinates from Bregma (anteroposterior, lateral, ventral) were, FC, 1.6 mm, 1.3 mm, 1.6 mm, vH, −2.8 mm, 3.0 mm, 4 mm, and DRN, −4.5 mm, 0 mm, 3.5 mm. Animals were allowed to recover from surgery overnight and were continuously perfused with artificial cerebrospinal fluid (147 mM NaCl, 3.5 mM KCl, 1.26 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 1.0 mM NaH_2PO_4, pH 7.4 ± 0.2) the next day. Dialysate samples were collected every 15 min for the FC and vH (flow rate, 1.5 μl/min) and every 30 min for the DRN (flow rate, 0.5 μl/min). Extracellular 5-HT levels were measured using a high-performance liquid chromatography system [limit of sensitivity ≈ 1 fmol per sample (signal-to-noise ratio = 2)]. In each experiment, after 1 h of stabilization, four samples were collected to measure basal 5-HT values (means ± S.E.M.). The administration of pharmacological agents occurred at t = 0 and subsequent fractions were collected. SP (5–200 ng) was directly infused into the DRN [0.1 μl/min for 2 min via a microinjector (Harvard Appartus, France)], by means of a silica catheter glued to the microdialysis probe. WAY100635 (100 μM) (Guilloux et al., 2006) or 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM) (Tao et al., 1997) was injected via the probe after their dissolving in the artificial cerebrospinal fluid. The exact probe locations in brains were determined according to Bert et al. (2004).

Drugs. SP was obtained from Neosystem (Strasbourg, France). GR205171 was a gift from GlaxoSmithKline (Harlow, UK). WAY100635 and DNQX were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Data Analysis and Statistics. Baseline levels of 5-HT were calculated by averaging the levels measured in the first four samples collected before treatment. After administration of the treatment, the 5-HT content of individual dialysate samples was measured and expressed as a percentage of the baseline mean. The summed effects of each treatment over the course were measured by determining the area under the curve (AUC; mean ± S.E.M.) values for 5-HT outflow during the 0- to 120-min period after treatment. Comparisons of the effects of the different doses of SP on extracellular 5-HT levels were performed on AUC values by using a one-way ANOVA followed by a Fisher's protected least-significant difference post hoc test. The overall effects of "drug pretreatment and treatment" as main factors was assessed by using a two-way ANOVA followed by Fisher's protected least-significant difference post hoc test when appropriate. Finally, a Student's *t* test was used to compare two experimental groups, in particular the effects of SP versus vehicle in $NK1^{-/-}$ and 5-$HT_{1A}{}^{-/-}$ mutant mice.

Results and Discussion

Intraraphe Injection of Substance P Reduced Cortical Extracellular 5-HT Levels. In NK1 wild-type (+/+) control mice, the intraraphe (i.r.) injection of SP (50 and 200 ng) dose-dependently reduced extracellular levels of 5-HT in

TABLE 1
Basal 5-HT levels across all wild-type and mutant mice
Data are means ± S.E.M. of extracellular 5-HT levels. The numbers of determinations are in parentheses.

	FC	DRN
	fmol/20 μl	*fmol/10 μl*
NK1		
+/+ Mice	9.3 ± 0.9 (*n*=78)	N.D.
−/− Mice	10.6 ± 0.9 (*n*=14)	N.D.
5-HT_{1A}		
+/+ Mice	9.7 ± 0.8 (*n*=68)	13.7 ± 2.1 (*n* = 27)
−/− Mice	10.1 ± 0.7 (*n*=18)	N.D.

N.D., not determined.

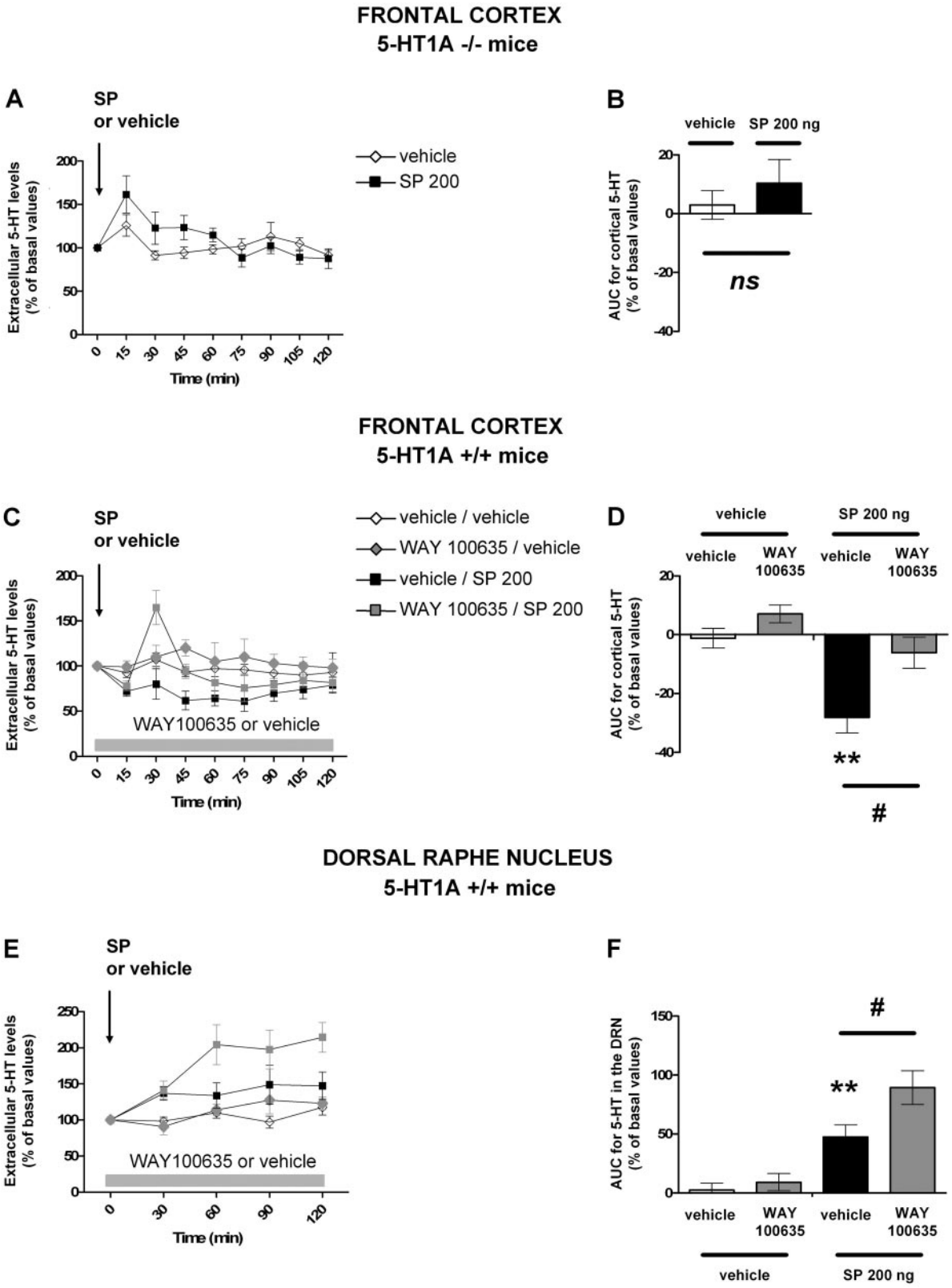

Fig. 2. Effects of genetic and pharmacological inactivation of 5-HT_{1A} receptors on substance P-induced changes in 5-HT outflow in the frontal cortex and dorsal raphe nucleus. A, C, and E, data are means ± S.E.M. of extracellular 5-HT levels expressed as percentages of basal values (arrows show the time of vehicle or SP injection, whereas the gray line indicates the duration of intraraphe perfusion of vehicle or WAY100635 through reverse dialysis). B, D, and F, data are AUC (means ± S.E.M.) values calculated for the amount of 5-HT outflow. B, a Student's *t* test revealed no significant difference between the effect of vehicle and SP (200 ng) on extracellular 5-HT levels in the FC of 5-$HT_{1A}^{-/-}$ mice. D, a two-way ANOVA on AUC 5-HT ext values revealed a significant main effect of pretreatment (vehicle or WAY100635, 100 μM) [$F_{(1,25)} = 3.8$; $p < 0.05$] and treatment (vehicle or SP) [$F_{(1,25)} = 15.6$; $p < 0.001$] factors, but no interaction between these two independent variables [$F_{(1,25)} = 1.2$; $p = 0.27$] in the FC of 5-$HT_{1A}^{+/+}$ mice. F, A two-way ANOVA on AUC 5-HText values revealed a significant main effect of pretreatment (vehicle or WAY100635) [$F_{(1,24)} = 5.5$; $p < 0.05$] and

the frontal cortex (Fig. 1A and B). These findings concur with in vivo electrophysiological data in which the neuronal activity of nearly 80% of DRN 5-HT neurons in rats was inhibited by the local microinjection of SP (Valentino et al., 2003). It is noteworthy that we found that the i.r. injection of the highest dose of SP (200 ng) failed to alter extracellular levels of 5-HT in the ventral hippocampus compared with the corresponding group of vehicle-treated wild-type mice (AUC values, $-0.7 \pm 6.2\%$ versus $3.1 \pm 5.1\%$, respectively; $P > 0.05$). It seems, therefore, that SP regulates the DRN activity in a region-dependent manner as previously proposed. A corollary of these intriguing findings is that the efferents of the DRN may differentially drive the activity of 5-HT neurons projecting to different forebrain structures, depending on whether these cells are activated or inhibited by SP (Valentino and Commons, 2005). From our neurochemical data, it can be postulated that SP inhibits the subpopulation of DRN 5-HT neurons projecting to the FC. In contrast, the lack of effect of i.r. injection of SP on extracellular 5-HT levels in the ventral hippocampus suggests that the latter region is not innervated by the DRN 5-HT neurons. This is in agreement with previous studies, which provided both anatomical and pharmacological evidence (Molliver, 1987; Kreiss and Lucki, 1994). Another possible explanation would be that the ventral hippocampus receives both types of innervations (i.e., neurons excited and inhibited by SP), leading to a blunted response. Although this would be important to address in future investigations, it is important to emphasize that the precise injection of drugs in mice is technically difficult, and we have to consider the possibility that SP diffused outside the DRN injection site. The median raphe nucleus, an adjacent area expressing NK1 receptors (Saffroy et al., 2003), is a region of interest as it sends serotonergic projections to the hippocampus. Nevertheless, in contrast to the present results, initial observations indicate that the injection of SP into the median raphe nucleus produces an excitatory effect on 5-HT turnover and probably neurotransmission in the hippocampus (Forchetti et al., 1982), strongly suggesting that, under our experimental conditions, the diffusion of SP was restricted to the DRN.

Involvement of NK1 Receptors in the Cortical Effect of Intraraphe Injection of Substance P. SP preferentially binds to and activates neurokinin 1 (NK1) receptors. However, it is well established that this neuropeptide may act as an agonist on NK2 and NK3 receptor subtypes, albeit with lower affinities (Maggi et al., 1993). Pharmacological and genetic experiments were thus conducted to assess whether the above inhibitory effects of SP on cortical 5-HT release were specifically mediated by NK1 receptors. In $NK1^{+/+}$ mice, the i.r. injection of SP (200 ng) did not modify cortical extracellular 5-HT levels when the potent and selective NK1 receptor antagonist GR205171 (30 mg/kg i.p.) was given 30 min before (Fig. 1, C and D). The dose of GR205171 was chosen on the basis of its capacity to block rat brain NK1 receptors in vivo (Rupniak et al., 2003). Similar results have been obtained using a genetic approach. Indeed, in contrast to $NK1^{+/+}$ mice, we showed that $NK1^{-/-}$ mutant mice were insensitive to the i.r. injection of SP (200 ng) on cortical 5-HT release (Fig. 1, E and F). It is noteworthy that neither the pharmacological nor the genetic inactivation of NK1 receptors altered basal extracellular levels of 5-HT in the FC of the mice (Table 1; Fig. 1, D and F). These results concur with recent microdialysis experiments performed in awake mice (Zocchi et al., 2003; Guiard et al., 2004), suggesting a lack of tonic regulation of 5-HT transmission by SP. It is noteworthy that NK1 receptor antagonists have been reported to be efficient anxiolytic and antidepressant agents in several animal models (Chahl, 2006). Because SP and 5-HT coexist in a substantial part of the neuronal DRN population in human and rodent brains (Chan-Palay et al., 1978; Sergeyev et al., 1999), it is possible that 5-HT neurons may regulate the release of SP in stressful conditions (Ebner et al., 2004). Thus, the present results demonstrate that an increase in endogenous SP levels in the DRN (mimicked here by its local injection) specifically activates NK1 receptors, subsequently inhibiting cortical 5-HT neurotransmission. Additional work is now required to address how this particular effect of i.r. injection of SP affects depressive-like symptoms such as anhedonia or despair in various animal paradigms. Such studies could further support the hypothesis that the putative antidepressant activity of NK1 receptors antagonists is related, at least in part, to the blockade of SP neurotransmission within the DRN.

Indirect involvement of Somatodendritic 5-HT_{1A} Autoreceptors in the Cortical Effects of Intraraphe Injection of Substance P. Given that SP was described as an excitatory neuropeptide, we raised the possibility that its neurochemical effects on cortical 5-HT release might indirectly recruit an inhibitory component in the DRN. Because somatodendritic 5-HT_{1A} autoreceptors play that role in the DRN (Hjorth et al., 2000), the i.r. injection of SP was evaluated in 5-$HT_{1A}^{-/-}$ mice. In these mutant mice, SP (200 ng) failed to modify extracellular levels of 5-HT in the FC (Fig. 2, A and B). However, by using a genetic approach (e.g., constitutive disruption of 5-HT_{1A} receptors by homologous recombination of the gene), we cannot definitively state whether presynaptic (in the DRN) rather than postsynaptic (in the FC) 5-HT_{1A} receptors were involved in the inhibitory effects of SP on cortical 5-HT release. Indeed, evidence does exist that 5-HT_{1A} autoreceptors located in the prefrontal cortex are involved in a distal control of DRN 5-HT neuronal activity (Celada et al., 2001). To determine whether there is a preferential activation of presynaptic 5-HT_{1A} receptors in the SP response, the selective 5-HT_{1A} receptor antagonist WAY100635 was perfused for 2 h into the DRN by reverse microdialysis in 5-$HT_{1A}^{+/+}$ wild-type mice as described previously (Guilloux et al., 2006). In the FC of 5-$HT_{1A}^{+/+}$ mice, pretreatment with the selective 5-HT_{1A} receptor antagonist

treatment (vehicle or SP) [$F_{(1,24)} = 35.5$; $p < 0.001$] factors, but no interaction between these two factors [$F_{(1,24)} = 2.7$; $p = 0.1$] in the DRN of 5-$HT_{1A}^{+/+}$ mice. Differences between groups of mice were determined by Fisher's post hoc test. *ns*, not statistically significant. **, $P < 0.01$ compared with vehicle-treated mice; #, $P < 0.05$ compared with vehicle/SP treated wild-type mice. The number of determinations (n) and means ± S.E.M. of baseline 5-HT levels, expressed as femtomoles per sample for each experimental group in the FC, were: vehicle ($n = 9$; 9.8 ± 0.7), SP 200 ($n = 9$; 10.4 ± 0.7) (Fig. 2A); vehicle/vehicle ($n = 7$; 10.1 ± 0.9), WAY100635/vehicle ($n = 10$; 10.2 ± 0.8), vehicle/SP 200 ($n = 11$; 8.9 ± 1.4), and WAY100635/SP 200 ($n = 10$; 10.9 ± 1.1) (Fig. 2C); and in the DRN, vehicle/vehicle ($n = 5$; 13.9 ± 2.1), WAY100635/vehicle ($n = 5$; 16.1 ± 2.5), vehicle/SP 200 ($n = 8$; 14.3 ± 1.7), and WAY100635/SP 200 ($n = 7$; 12.5 ± 1.8) (Fig. 2E). No significant differences were detected in baseline levels between experimental groups for individual experiments.

WAY100635 (100 μM, intraraphe), but not with the vehicle, significantly blocked the effects of SP injection (200 ng) on extracellular levels of 5-HT (Fig. 2, C and D). These results are consistent with the findings that both systemic and i.r. injection of WAY100635 attenuated the predominantly inhibitory effects of SP on the DRN 5-HT neuronal activity (Valentino et al., 2003). In marked contrast to the effects observed in the FC, we showed here for the first time that the i.r. injection of SP (200 ng) increased extracellular levels of 5-HT in the DRN compared with the vehicle-treated group in $5\text{-HT}_{1A}{}^{+/+}$ mice, the latter effect being potentiated by WAY100635 (Fig. 2, E and F). The opposite effects of SP on extracellular 5-HT outflow in the DRN and FC appear to be somewhat equivocal. Nevertheless, they allowed anticipating that SP-induced decreases in cortical 5-HT release resulted from an overactivation of inhibitory 5-HT_{1A} autoreceptors in the DRN. Previous studies in rats reported that relatively high extracellular 5-HT concentrations were required in the DRN to reduce forebrain 5-HT release through the activation of 5-HT_{1A} autoreceptors (Romero and Artigas, 1997; Tao and

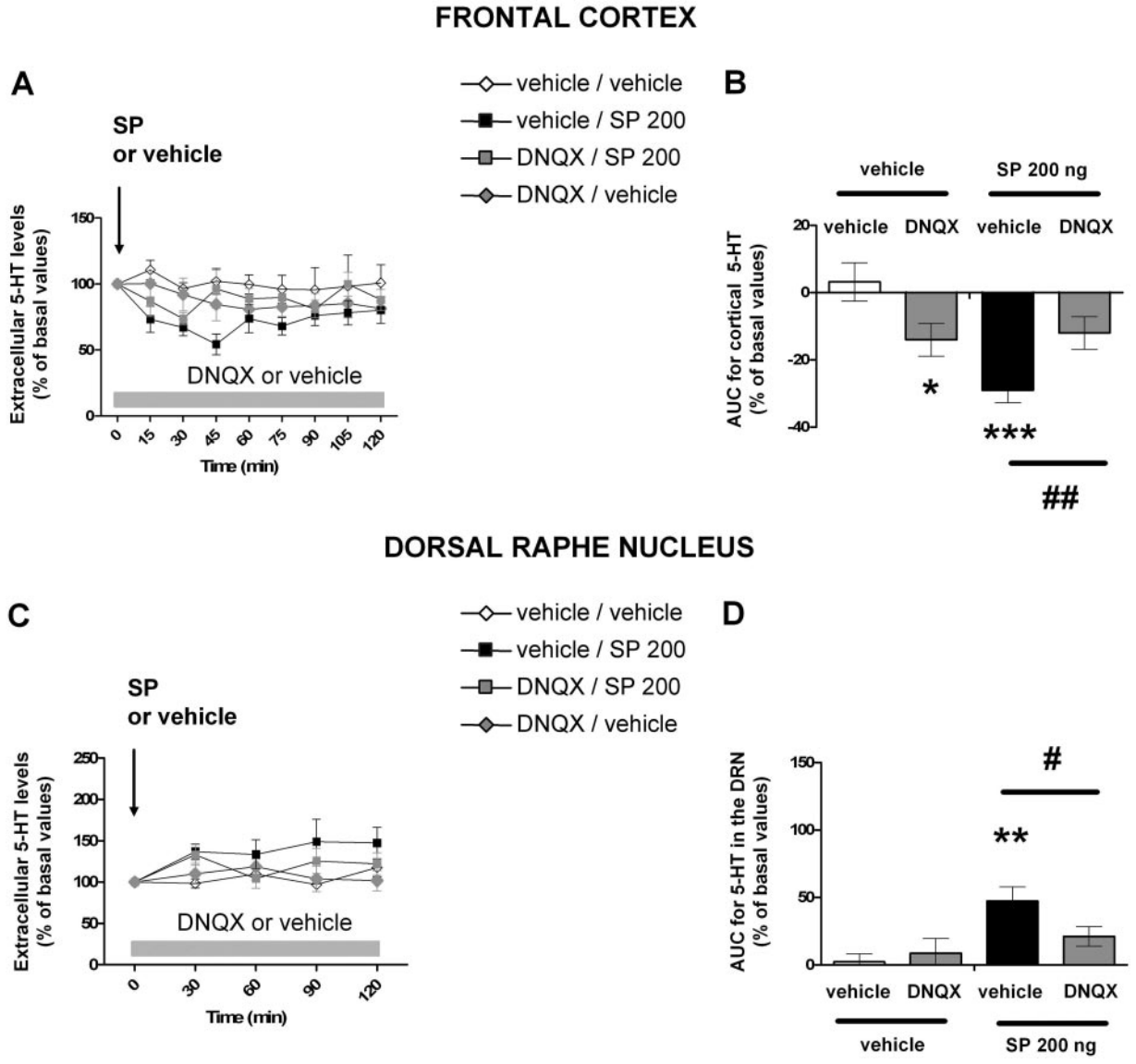

Fig. 3. Effects of pharmacological inactivation of AMPA/kainate receptors on substance P-induced changes outflow in the frontal cortex and dorsal raphe nucleus in wild-type mice. A and C, data are means ± S.E.M. of extracellular 5-HT levels expressed as percentages of basal values (arrows show the time of drug injection, whereas the gray line indicates the duration of intraraphe perfusion of vehicle DNQX through reverse dialysis). B and D, data are AUC (means ± S.E.M.) values calculated for the amount of 5-HT outflow. B, an overall two-way ANOVA on AUC 5-HText values revealed no significant effect of pretreatment factor (vehicle or DNQX, 10 μM) [$F_{(1,26)} = 0.0002$; $p = 0.9$], but a significant effect of treatment factor (vehicle or SP) [$F_{(1,26)} = 9.7$; $p < 0.01$] and a significant interaction between these two factors [$F_{(1,25)} = 12.1$; $p < 0.01$] in the FC of wild-type mice. D, an overall two-way ANOVA on AUC 5-HText values revealed no significant effect of pretreatment factor (vehicle or DNQX) [$F_{(1,20)} = 0.8$; $p = 0.3$], but a significant effect of treatment factor (vehicle or SP) [$F_{(1,20)} = 9.5$; $p < 0.01$] and a significant interaction between these two factors [$F_{(1,20)} = 4.4$; $p < 0.05$] in the DRN of wild-type mice. Differences between groups of mice were determined by Fisher post hoc test. *, $P < 0.05$; **, $P < 0.01$ and ***, $P < 0.001$ compared with vehicle-treated mice; #, $P < 0.05$ and ##, $P < 0.01$ compared with vehicle/SP treated mice. The number of determinations (n) and means ± S.E.M. of baseline 5-HT levels espressed as femtomoles per sample for each experimental group in the FC were: vehicle/vehicle (n = 7; 10.1 ± 0.9), DNQX/vehicle (n = 6; 9.9 ± 1.1), vehicle/SP 200 (n = 11; 9.4 ± 0.6), and DNQX/SP 200 (n = 6; 8.7 ± 1.1) (Fig. 3A); and in the DRN were: vehicle/vehicle (n = 5; 13.9 ± 2.1), DNQX/vehicle (n = 5; 15.1 ± 2.7), vehicle/SP 200 (n = 8; 14.3 ± 1.7), and DNQX/SP 200 (n = 6; 12.9 ± 2.1) (Fig. 3C). No significant differences were detected in baseline levels between experimental groups for individual experiments.

Auerbach, 2000). No direct evidence supporting these observations has been provided in mice. On the contrary, in a recent study, we reported that a 2-fold decrease in extracellular 5-HT outflow in the DRN was sufficient to trigger an equivalent increase in the FC in mice (Guiard et al., 2004). Activation of DRN 5-HT_{1A} autoreceptors in response to SP might contribute to the inhibition of cortical 5-HT release. It is noteworthy that despite evidence indicating a tonic inhibitory effect of DRN 5-HT_{1A} autoreceptors on 5-HT neuronal activity (Haddjeri et al., 2004), we observed that neither the pharmacological nor the genetic inactivation of 5-HT_{1A} autoreceptors altered the basal extracellular 5-HT levels in the frontal cortex and the DRN (Table 1, Fig. 2, B and D). These findings are in agreement with initial microdialysis studies performed in mice at somatodendritic (Bortolozzi et al., 2004; Guilloux et al., 2006) and terminal levels (He et al., 2001; Knobelman et al., 2001; Guilloux et al., 2006).

Role of Excitatory Amino Acid Receptors in the Substance P-Induced Effect. The above findings (i.e., SP-induced inhibition of cortical 5-HT release through an overactivation of DRN 5-HT_{1A} autoreceptors) may occur through a local elevation of endogenous 5-HT. According to Liu and Aghajanian (2002), such an elevation of 5-HT outflow occurring in the DRN could be attributable to a previous increase in glutamate transmission in the DRN. Consistent with this assumption are the observations that the DRN is endowed with a rich population of NK1 receptors especially dense on glutamate interneurons (Commons and Valentino, 2002; Liu et al., 2002, Commons et al., 2003). A last series of experiments was conducted to determine the putative involvement of glutamate in the neurochemical response to i.r. injection of SP. In our experimental conditions, we showed that the glutamate AMPA/kainate receptor blocker DNQX alone produced a slight but significant decrease in cortical 5-HT release in $NK1^{+/+}$ mice. Despite its own effect, DNQX (10 μM, intraraphe) significantly attenuated the SP-induced decrease in cortical 5-HT levels (Fig. 3, A and B). This strongly suggests that the disinhibitory effect of the AMPA/kainate receptor antagonist could be ascribed to a specific involvement of glutamate in the SP response. In line with this assumption, the increase in 5-HT outflow induced by i.r. injection of SP (200 ng) was also blocked by DNQX (10 μM, i.r.) in the DRN in $NK1^{+/+}$ mice (Fig. 3, C and D), whereas DNQX alone had no effect on DRN extracellular 5-HT levels as demonstrated previously (Tao et al., 1997; Tao and Auerbach., 2003). Based on these results, it can be postulated that increased glutamate release in the DRN would occur after i.r. injection of SP. However, it is important to emphasize that the i.r. injection of glutamate receptor agonists enhances the release of 5-HT at both somatodendritic and nerve terminals levels (Tao and Auerbach, 2000), whereas in the present study, the i.r. injection of SP-produced opposite effects in the DRN and FC. Because SP-induced stimulation of glutamate transmission in the DRN is a short-lasting effect (return to baseline ~10 min after injection; Liu et al., 2002), it is possible that we failed to detect any increases in 5-HT cortical release using intracerebral microdialysis. It is also possible that the elevated pool of extracellular 5-HT levels in the DRN of wild-type mice injected with SP did not solely originate from DRN 5-HT neuronal cell bodies or dendrites. Because the DRN receives serotonergic innervations from the other raphe nuclei (Tischler and Morin, 2003), the release of 5-HT may also result from the stimulation of AMPA/kainate receptors on serotonergic afferents in the DRN (Fig. 4). Alternative mechanisms, such as direct activation of NK1 receptors located on the 5-HT cell bodies, could also account for the inhibitory effect of SP on the serotonergic system. This hypothesis is supported by recent evidence showing that almost 30% of rats and mice DRN 5-HT neurons express NK1 receptors (Lacoste et al., 2006). Finally, NK1 receptors have been clearly identified on GABAergic neurons surrounding 5-HT cell bodies in the DRN and their activation might contribute, at least in part, toward SP-induced attenuation of cortical 5-HT neurotransmission.

In conclusion, our neurochemical data support the idea that increased brain SP levels, specifically in the DRN, may represent an important step in the pathophysiology of depression. In these conditions, the potential antidepressant effects of NK1 receptor antagonists (Chahl, 2006) could be related to their capacity to block or prevent the reported

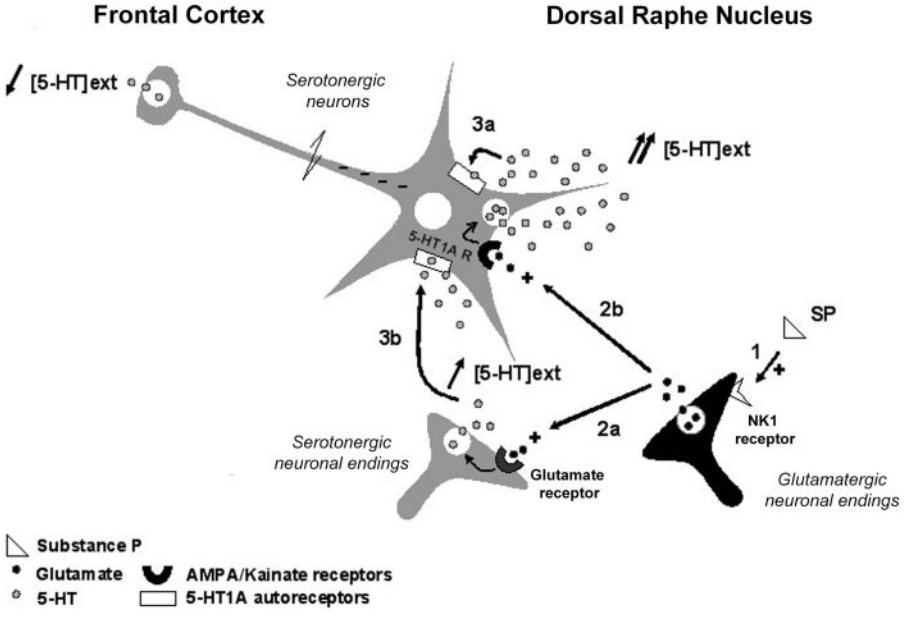

Fig. 4. Model of substance P regulation of 5-HT neurotransmission. In the DRN, SP activates NK1 receptors located on glutamatergic neurons (1) and produces glutamate release (black circles). (2) Because the DRN receives serotonergic innervations, the enhancement of glutamate release could stimulate 5-HT release (gray circles) through presynaptic glutamate AMPA/kainate receptors on serotonergic afferents in the DRN [2a]. As well, 5-HT release could result from the activation of AMPA/kainate receptor located on 5-HT cell bodies [2b]. (3) The excess of extracellular 5-HT levels in the DRN resulting from local activation of AMPA/kainate receptors triggers a delayed inhibition of cortical 5-HT release via 5-HT_{1A} autoreceptor over-activation.

effect of SP on cortical 5-HT transmission. However, it is important to mention that despite the great enthusiasm raised by the first placebo-controlled trials, no antidepressant or modest efficacy of NK1 receptor antagonists were reported in subsequent clinical studies, leading several pharmaceutical companies to discontinue their research program in this field (Czéh et al., 2006; Holtzheimer and Nemeroff, 2006). Additional studies are needed to further characterize the real impact of tachykinins on the 5-HT system, as well as the pathophysiology and treatments of depression. In particular, based on the present data, it can be proposed that abnormal SP neurotransmission in the DRN is involved in the inadequate response to the selective serotonin reuptake inhibitors (e.g., long delay of action and/or resistance to the treatment) in some patients. Consequently, rather than being used as monotherapy, NK1 receptor antagonists could conceivably be prescribed as augmentation agents in combination with a traditional antidepressant (Ryckmans et al., 2002; Guiard et al., 2004). This should arouse our attention for future clinical investigations.

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Address correspondence to: Alain M. Gardier, Univ Paris-Sud EA 3544, Fac. Pharmacie, Chatenay-Malabry cedex F92296, France. E-mail: alain.gardier@u-psud.fr