Frontocortical 5-HT$_4$ Receptors Exert Positive Feedback on Serotonergic Activity: Viral Transfections, Subacute and Chronic Treatments with 5-HT$_4$ Agonists

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Background: *We recently identified a facilitory control exerted by serotonin$_4$ (5-HT$_4$) receptors on the in vivo firing activity of dorsal raphé nucleus (DRN) serotonergic (5-HT) neurons. However, these findings were based on acute administrations of 5-HT$_4$ receptor agonists and antagonists, which were active only in a subpopulation of 5-HT neurons. We had no evidence that this influence was significant when considering the entire DRN, nor if it was persistent after chronic treatments. In addition, the poor distribution of 5-HT$_4$ receptors within the DRN raised the question of the neuroanatomical bases underlying this control.*

Methods and Results: *Here we show that the subacute intraperitoneal (IP) injection of the 5-HT$_4$ receptor agonists prucalopride (2.5 mg/kg) and RS 67333 (1.5 mg/kg) 30 minutes before the beginning of recordings augment the mean firing rate of DRN neurons by 40% and 66%, respectively. These increases remain stable when the compounds are administered continuously during 3 and 21 days; the effects of the 3-day treatment are blocked by the 5-HT$_4$ receptor antagonist GR 125487 (1000 μg/kg, intravenous [i.v.]). In addition, stereotaxic microinjections of herpes simplex viruses, transformed to overexpress 5-HT$_4$ receptors, increase DRN 5-HT neuronal mean activity when performed in the medial prefrontal cortex (mPFC) but not in the striatum or in the hippocampus.*

Conclusions: *This finding suggests the existence of a 5-HT$_4$-dependent activation of DRN that may involve the mPFC, unveiling the 5-HT$_4$ receptor as a putative player in the physiopathology of several disorders related to central 5-HT dysfunction*

Key Words: 5-HT$_4$ receptors, medial prefrontal cortex, serotonergic neurons, dorsal raphé, viral-mediated gene transfer, electrophysiology

The importance of dorsal raphé nucleus (DRN) serotonergic (5-HT) neurons is now well established in the pathophysiology of major depression (Mongeau et al 1997; Artigas et al 2002; Fabre and Hamon 2003). So far, all antidepressant treatments increase the efficacy of 5-HT transmission at the postsynaptic levels (Haddjeri et al 1998; Blier and de Montigny 1999). This is the case of selective serotonin reuptake inhibitors (SSRIs), the most commonly used antidepressants. Unfortunately, the initial elevation of 5-HT concentration triggered by such agents induces the stimulation of inhibitory 5-HT$_{1A}$ autoreceptors within the DRN, counteracting the facilitation of 5-HT transmission related to terminal reuptake blockade (Mongeau et al 1997; Blier and de Montigny 1999). The existence of this presynaptic effect is believed to be responsible for the 3 to 6 weeks delay before the onset of the antidepressant's therapeutic action, as this period corresponds to the time required for 5-HT$_{1A}$ autoreceptors to desensitize (Haddjeri et al 1998; Blier and de Montigny 1999). Based on these observations, it has been proposed that a direct facilitation of 5-HT firing rate should be a requirement for a faster onset of antidepressant action (Blier 2001). Interestingly, we recently identified a tonic and phasic facilitory control exerted by 5-HT$_4$ receptors on the firing activity of DRN 5-HT neurons, based on the effects of systemically administered 5-HT$_4$ pharmacological compounds in anaesthetized rats (Lucas and Debonnel 2002). We found that the activity of DRN 5-HT neurons was increased by 5-HT$_4$ receptor agonists and decreased by 5-HT$_4$ receptor antagonists.

Two distinct issues were addressed in the present study. First, the facilitation of 5-HT neuronal activity was observed after an acute stimulation of 5-HT$_4$ receptors and in only one half of the recorded neurons (labeled as "responders" in our previous report) (Lucas and Debonnel 2002). We had no evidence that this effect was significant when considering the entire DRN and that it persisted after sustained treatments. Second, the expression of 5-HT$_4$ receptors (both messenger RNA [mRNA] and protein) is very low within the DRN itself (Waeber et al 1994; Vilaro et al 1996), raising the question of the neuroanatomical bases underlying the 5-HT$_4$–mediated control. The two aspects were addressed separately, based on the characteristics of the control described above. First, the phasic component permitted us to assess the effect of subacute and chronic administrations of 5-HT$_4$ receptor agonists. Second, we postulated that if the tonus exerted by endogenous 5-HT at 5-HT$_4$ receptors could be selectively enhanced in the brain area(s) from which the control originates, 5-HT neuronal activity should be increased in the DRN. To achieve such an enhancement, we performed stereotaxic injections of herpes simplex virus (HSV) vectors, transformed with an amplicon allowing the overexpression of 5-HT$_4$ receptors (HSV–5-HT$_4$). Similar viral transfections have already been used to facilitate the expression of various proteins, such as transcription factors (Barrot et al 2002) or other 5-HT receptor types (Neumaier et al 2002). Both in the presence of agonists or after viral transfections, successive single-cell extracellular recording tracks were performed along the DRN of anesthetized rats to assess the effect of treatments on the global, mean activity of 5-HT neurons.

Methods and Materials

Animals

Experiments were carried out in male Sprague-Dawley rats (Charles River, St-Constant, Québec, Canada) weighing 270 g to

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300 g and kept under standard laboratory conditions. Experiments were done in compliance with the Canadian Council and Animal Care's guidelines for the Use of Experimental Animals.

Generation of HSV Construct for 5-HT$_4$ Receptors

Briefly, complementary DNA (cDNA) fragment comprising the open reading frame of 5-HT$_4$ gene (obtained from the full-length 5-HT$_{4a}$ splice variant) was inserted into the HSVPrpUC amplicon (from Dr. R.L. Neve's laboratory). The amplicon construct was packaged with helper 5dl1.2, purified on a sucrose gradient, pelleted, and resuspended in 10% sucrose. Transgene expression was regulated by HSV immediate-early gene promoter IE4/5. The HSV-LacZ constructs were used as control constructs for the viral-mediated gene delivery.

Pharmacological Treatments

The selective 5-HT$_4$ receptor agonists prucalopride (Briejer et al 2001) and RS 67333 (Eglen et al 1995) were administered either subacutely (i.e., 30 minutes before the beginning of recordings) or continuously through the use of osmotic minipumps (Alza, Palo Alto, California) during 3 or 21 days. The selective 5-HT$_4$ receptor antagonist GR 125487 (Gale et al 1994) was also used to confirm the involvement of 5-HT$_4$ receptors in the effects of the agonists.

All the three different 5-HT$_4$ pharmacological agents were diluted in a solution of physiological saline (sodium chloride [NaCl] .9%) and administered in a dose range known to induce selective physiological responses (Lucas and Debonnel 2002; Lamirault and Simon 2001; Porras et al 2002). In subacute experiments, prucalopride (2.5 mg/kg), RS 67333 (1.5 mg/kg), or their vehicle was administered intraperitoneal (IP) 30 minutes before the beginning of recordings. For chronic treatments (both 3 and 21 days), prucalopride (2.5 mg/kg/day), RS 67333 (1.5 mg/kg/day), or their vehicle was delivered through osmotic minipumps (Alza, Palo Alto, California) inserted subcutaneously. Electrophysiological recordings were performed with the minipumps still in place. Results of vehicle-treated (IP and via minipumps) animals were not statistically different from each other, and data were pooled and randomized to constitute the control groups displayed in Figures 1 and 2. In a separate set of experiments, GR 125487 (1000 μg/kg) was tested in animals treated for 3 days with either prucalopride or RS 67333. Two DRN tracks were first performed, then GR 125487 was administered intravenous (i.v.) (via a lateral vein of the tail) and two to three additional tracks were performed, starting 30 minutes after the injection. All drug dosages refer to the free base.

Viral-Mediated Gene Transfer

The injections were done in either of the two distinct groups of brain structures in which 5-HT$_4$ receptors are already present. One group parallels ascending dopaminergic (DA) pathways and is essentially represented by ventral and dorsal striatum and olfactory bulbs (Waeber et al 1994; Compan et al 1996). The other group includes limbic areas linked with emotional control, mainly the CA$_1$ and CA$_3$ subregions of the hippocampal formation, but significant levels are also found within the medial prefrontal cortex (mPFC) (Waeber et al 1994). Stereotaxic surgery was performed as described (Barrot et al 2002; Neumaier et al 2002). Herpes simplex virus vectors (10^7 infectious units per mL) were injected bilaterally into the targeted structures. For each side, two sites of injections were selected along the dorsal-ventral axis to ensure a maximal diffusion of the particles and to target both the dorsal and ventral parts in the case of striatum.

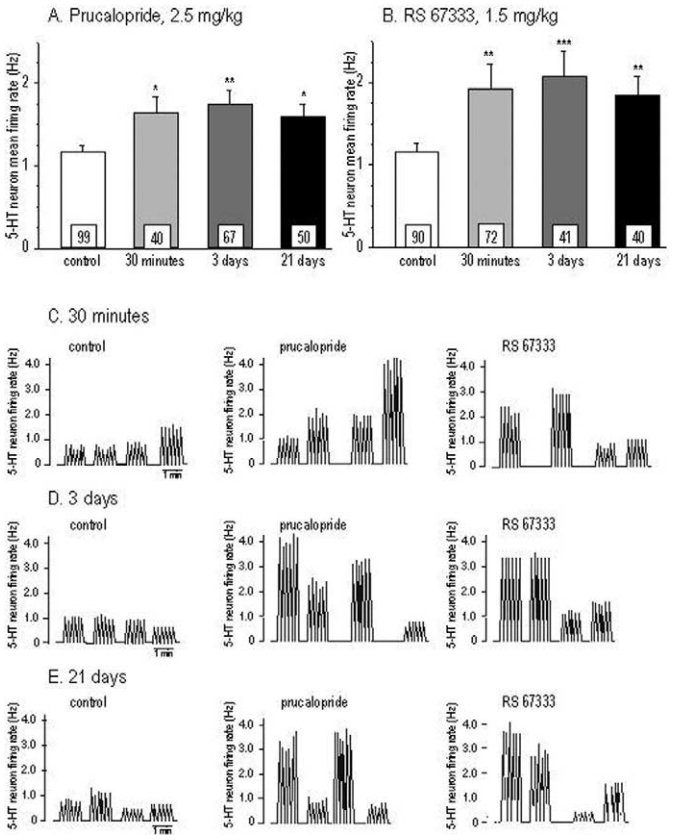

Figure 1. Effect of subacute, 3-day and 21-day chronic treatments with **(A)** prucalopride (2.5 mg/kg) and **(B)** RS 67333 (1.5 mg/kg) on the mean firing rate of DRN 5-HT neurons. In subacute conditions, the agonists were administered IP 30 minutes before the beginning of recordings. For chronic treatments, prucalopride and RS 67333 were administered via osmotic minipumps inserted subcutaneously; in this case, dose refers to the total daily dosage. The number at the bottom of the column represents the total of cells recorded for each group. *$p < .05$, **$p < .01$, and ***$p < .001$ versus control group, Dunnett test. **(C)** Integrated firing rate histogram showing samples of DRN descents in subacute conditions, **(D)** after a 3-day chronic treatment and **(E)** after a 21-day chronic treatment. Left, center, and right panels illustrate the tracks performed in control conditions in the presence of prucalopride and of RS 67333, respectively. DRN, dorsal raphé nucleus; 5-HT, serotonin.

Injections consisted of 1 μL per site over 1 minute in the mPFC and striatum and 2 minutes in the hippocampus. Coordinates (relative to bregma for AP and L and to dura for V) were (in mm): AP: +3.4, L: ±.5, V: 4.8 and 4.4 for mPFC; AP: +.7, L: ±3, V: 6.2 and 5.8 for striatum; and AP: -4.8, L: ±4, V: 3.2 and 2.8 for hippocampus. Injection placements and expression were checked by in situ hybridization on each animal at the end of the experiment. Based on the previously determined time course of transgene expression (Barrot et al 2002; Neumaier et al 2002), animals were tested for electrophysiological response on day 3 postinjection. In each cerebral structure, "sham" operations (descent of cannula without injection) were also performed; since these results were not statistically different from each other, data were pooled and randomized to constitute the control group (labeled "sham" in Figure 4A).

Extracellular Recordings of DRN 5-HT Neurons

Recordings were performed using single-barreled glass micropipettes. Electrodes were filled with a 2 mol/L NaCl solution saturated