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STIMULATION OF SEROTONIN2C RECEPTORS ELICITS ABNORMAL ORAL MOVEMENTS BY ACTING ON PATHWAYS OTHER THAN THE SENSORIMOTOR ONE IN THE RAT BASAL GANGLIA

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Abstract—Serotonin2C (5-HT_{2C}) receptors act in the basal ganglia, a group of sub-cortical structures involved in motor behavior, where they are thought to modulate oral activity and participate in iatrogenic motor side-effects in Parkinson's disease and Schizophrenia. Whether abnormal movements initiated by 5-HT_{2C} receptors are directly consequent to dysfunctions of the motor circuit is uncertain. In the present study, we combined behavioral, immunohistochemical and extracellular single-cell recordings approaches in rats to investigate the effect of the 5-HT_{2C} agonist Ro-60-0175 respectively on orofacial dyskinesia, the expression of the marker of neuronal activity c-Fos in basal ganglia and the electrophysiological activity of substantia nigra pars reticulata (SNr) neuron connected to the orofacial motor cortex (OfMC) or the medial prefrontal cortex (mPFC). The results show that Ro-60-0175 (1 mg/kg) caused bouts of orofacial movements that were suppressed by the 5-HT_{2C} antagonist SB-243213 (1 mg/kg). Ro-60-0175 (0.3, 1, 3 mg/kg) dose-dependently enhanced Fos expression in the striatum and the nucleus accumbens. At the highest dose, it enhanced Fos expression in the subthalamic nucleus, the SNr and the entopeduncular nucleus but not in the external globus pallidus. However, the effect of Ro-60-0175 was mainly associated with associative/limbic regions of basal ganglia whereas subregions of basal ganglia corresponding to sensorimotor territories were devoid of Fos labeling. Ro-60-0175 (1–3 mg/kg) did not affect the electrophysiological activity of SNr neurons connected to the OfMC nor their excitatory-inhibitory-excitatory responses to the OfMC electrical stimulation. Conversely, Ro-60-0175 (1 mg/kg) enhanced the late excitatory response of SNr neurons evoked by the mPFC electrical stimulation. These results suggest that oral dyskinesia induced by 5-HT_{2C} agonists are not restricted to aberrant signalling in the orofacial motor circuit and demonstrate discrete modifications in associative territories.

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Abbreviations: DL, dorsolateral; DM, dorsomedial; EPN, entopeduncular nucleus; GP, globus pallidus; *m*-CPP, *meta*-chlorophenylpiperazine; mPFC, medial prefrontal cortex; OfMC, orofacial motor cortex; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VL, ventrolateral; VM, ventromedial.

Key words: single cell extracellular recordings, c-Fos, Ro-60-0175, orofacial motor cortex, substantia nigra pars reticulata, SB-243213.

It is well established that central serotonin (5-HT) system affects the activity of basal ganglia (Soubrié et al., 1984; Di Matteo et al., 2008), a group of sub-cortical structures involved in motor behavior. The 5-HT_{2C} receptor, a 5-HT receptor subtype belonging to the G-protein coupled receptor superfamily and largely expressed in basal ganglia (Clemett et al., 2000; Eberle-Wang et al., 1997; Pasqualetti et al., 1999), plays a prominent role in basal ganglia. The influence of 5-HT_{2C} receptors on mood and reward system through the modulation of limbic/associative territories of basal ganglia is documented (Grottick et al., 2000; Bubar and Cunningham, 2008). 5-HT_{2C} receptors can concomitantly generate abnormal motor movements including oral dyskinesia (Simansky et al., 2004), classically related to the sensorimotor circuit of basal ganglia (Marchand and Dilda, 2006). The influence of 5-HT_{2C} receptors in dyskinesia has been stressed in both humans and rodents (Gunes et al., 2008; Meltzer et al., 2003), but the way by which 5-HT_{2C} receptors could affect motor activity in basal ganglia has received little attention.

The link between the motor responses exerted by 5-HT_{2C} receptors and the sensorimotor territories of basal ganglia in rodents is not clear. Non selective 5-HT agonists such as *meta*-chlorophenylpiperazine (*m*-CPP) induce abnormal orofacial movements (Gong et al., 1992; Stewart et al., 1989; Wolf et al., 2005) that involve 5-HT_{2C} receptors of basal ganglia (Eberle-Wang et al., 1996; Plech et al., 1995). *m*-CPP alters indirect indexes of neuronal activity such as the expression of the proto-oncogene c-Fos in the associative/limbic territories of basal ganglia (Hackler et al., 2007; Singewald et al., 2003; Stark et al., 2006). Such findings raise the hypothesis that 5-HT_{2C} receptor stimulation induces dyskinesias related to associative/limbic areas. However, non-selective 5-HT_{2C} agonists also affect neuronal activity in sensorimotor areas (De Deurwaerdère and Chesselet, 2000; Stark et al., 2008) and indirect indexes of neuronal activity are not sufficient to discard modulatory effects in sensorimotor parts.

Glutamatergic neurons arising from various cortices innervate the subthalamic nucleus and the striatum, which send respectively glutamatergic and GABAergic neurons to SNr neurons. The stimulation of the orofacial motor cortex (OfMC) or the medial prefrontal cortex (mPFC) may lead to a triphasic response of SNr neurons topographi-

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cally and functionally associated with the lateral or the medial parts of basal ganglia, respectively (Kolomiets et al., 2003; Sgambato et al., 1997). The temporal and topographic sensitivity of this electrophysiological response combined with neuroanatomical markers and appropriate pharmacology could directly address whether 5-HT_{2C} receptor stimulation, thought to affect basal activity of SNr neurons (Invernizzi et al., 2007; Rick et al., 1995), would act on the sensorimotor territory to elicit oral dyskinesia.

Thus, combining behavioral, immunohistochemical and electrophysiological approaches, we have examined whether oral dyskinesia elicited by the stimulation of 5-HT_{2C} receptors in rats is associated with neuronal alteration in sensorimotor pathway of the basal ganglia. Oral dyskinesia was elicited by the preferential 5-HT_{2C} agonist Ro-60-0175 (Martin et al., 1998) alone or in the presence of the selective 5-HT_{2C} antagonist SB-243213 (Wood et al., 2001). The influence of Ro-60-0175 was studied in basal ganglia of awake rats by c-Fos expression. Its effect has been finally evaluated by single cell extracellular recordings of SNr neurons responding to OfMC stimulation and, in an additional analysis, to those responding to mPFC stimulation in urethane-anaesthetized rats.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (Charles River, Lyon, France) weighing 330–380 g were used. Animals were kept at constant room temperature (21±2 °C) and relative humidity (60%) with a 12-light/dark cycle (dark from 8 PM) and had free access to water and food. All animal use procedures conformed to International European Ethical Standards (86/609-EEC) and the French National Committee (*décret* 87/848) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Behavioural testing

Rats were brought to the behavioural room before the testing day in order to facilitate their adaptation to the conditions of the experiment. On the day of behavioural testing, each rat was placed in a square plastic chamber (30 cm diameter, 45 cm height). The 5-HT agonist Ro-60-0175 or its vehicle was administered intraperitoneally after a 30 min adaptation period and immediately prior to the onset of the behavioural observations. Thereafter, the rats were returned to the testing cage and observed continuously for 60 min for bouts of orofacial movements. The number of oral bouts was quantified by an observer blind to the drug schedule administration. Oral activity was defined as vacuous chewing, jaw tremors, and tongue darting that occurred without any reference to an evident physical target. Thus, oral activity occurring with feeding, grooming, liking or sniffing was not counted. Moreover, an interval of 2 s without oral activity was required to consider the oral bout fully terminated. The duration of each oral bout was not measured (Stewart et al., 1989; Eberle-Wang et al., 1996; De Deurwaerdère and Chesselet, 2000). After the one-hour period of behavioural testing, rats were returned to their cage.

Immunohistochemistry

Rats were deeply anesthetized with chloral hydrate (15%) and perfused transcardially with 0.01 M phosphate-buffered saline (PBS, pH 7.4, 37 °C) followed by ice-cold 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB). Brains were removed, post-fixed for 12 h in the same fixative (4 °C), and placed in PBS 0.1 M containing 0.03% sodium azide. Brains were stored at 4 °C. Forebrains were cut at 50 μm on a vibratome (VT1000S, Leica Instruments, France) in the coronal plane. Sections through the striatum, the nucleus accumbens, globus pallidus, entopeduncular nucleus, subthalamic nucleus and substantia nigra were collected in PBS (0.1 M, 4 °C) containing 0.03% sodium azide, and stored at 4 °C pending Fos immunohistochemistry.

After three washes in PBS 0.1 M (pH 7.4) at room temperature, sections were incubated (1 h) in PBS (0.1 M, pH 7.4) containing 3% normal donkey serum with 0.3% triton. Sections were transferred to PBS (0.1 M, 0.3% triton, pH 7.4) containing anti-Fos rabbit polyclonal antibody (1:8000; SC-52, Santa-Cruz Biotechnology, USA) for 40–44 h at 4 °C. After three washes in 0.1 M PBS (10 min each), sections were incubated for 2 h in PBS (0.1 M, 0.3% triton, pH 7.4) containing biotinylated goat anti-rabbit IgG (1:200, Amersham Biosciences, UK). After washing (three times in 0.1 M PBS), sections were incubated in avidin-biotin-peroxidase complex (2 h; ABC Vectastain Elite Kit, Vector laboratories, distributed by Biovalley SA, France) for subsequent staining with 3,3′-diaminobenzidine (0.05% w/v; Sigma, Saint-Quentin Fallavier, France) in 0.05 M Tris buffer saline (pH 7.6) containing 0.003% H_2O_2 (Sigma). Sections were finally washed three times in 0.05 M Tris saline buffer (pH 7.6). After processing, tissue sections were mounted onto gelatin-alum-coated slides, dehydrated in ascending concentration of ethanol, and coverslipped with Eukitt mounting medium (Calibrated Instruments, Hawthorne, NY, USA).

In some experiments, we used a double immunostaining of Fos and tyrosine hydroxylase (TH) proteins on mesencephalic slices to better delineate Fos labelling within the SNr more properly. We used the same Fos labelling procedure except that the staining using 3,3′-diaminobenzidine was intensified by adding nickel to medium (0.2%, g/v; grey colour). Thereafter, after three washes in PBS, sections were incubated in PBS (0.1 M, 0.3% triton, pH 7.4) anti-TH mouse polyclonal antibody (1:20,000; Incstar, France) overnight at room temperature. Then, after three washes, sections were incubated during 2 h in PBS (0.1 M, 0.3% triton, pH 7.4) containing biotinylated goat anti-mouse IgG (1:200, Amersham Biosciences, UK). After washes (three times in 0.1 M PBS), sections were incubated 2 h in avidin-biotin-peroxidase complex for subsequent staining with 3,3′-diaminobenzidine alone (brown colour) in PBS (0.1 M, pH 7.4) containing 0.003% H_2O_2. Subsequent procedures are the same than those described above.

Surgeries

In electrophysiological experiment, animals were anesthetized with urethane hydrochloride (1.5 g/kg i.p., supplemented by 50 mg/kg i.p. injections, Sigma-Alrich, France) and fixed in a conventional stereotaxic apparatus (Horsley Clarke apparatus, Unimécanique, Epinay sur Seine, France). Body temperature was monitored with a rectal probe and maintained at 37 °C with a homeothermic warming blanket (model 50-7061, Harvard Apparatus, Les Ulis, France).

Electrical stimulation of the orofacial motor and medial prefrontal cortices

Electrical stimulation of the orofacial/forelimb motor cortex (anterior [A]: 3.5; lateral [L]: 3 from the bregma; height [H]: 1.6 mm from the cortical surface), ipsilateral to the recording SNr site, was performed through a bipolar coaxial stainless steel electrode (SNEX-200, diameter 100 μm, tip-barrel distance, 500 μm, distributed by Phymep, Paris, France) positioned stereotaxically according to the atlas of Paxinos and Watson (1998). Electrical stimulation of the medial prefrontal cortex ([A]: 3.7; [L]: 0.6 from