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Research paper

Serotonin$_{2B}$ receptors in the rat dorsal raphe nucleus exert a GABA-mediated tonic inhibitory control on serotonin neurons

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ABSTRACT

The central serotonin$_{2B}$ receptor (5-HT$_{2B}$R) is a well-established modulator of dopamine (DA) neuron activity in the rodent brain. Recent studies in rats have shown that the effect of 5-HT$_{2B}$R antagonists on accumbal and medial prefrontal cortex (mPFC) DA outflow results from a primary action in the dorsal raphe nucleus (DRN), where they activate 5-HT neurons innervating the mPFC. Although the mechanisms underlying this interaction remain largely unknown, data in the literature suggest the involvement of DRN GABAergic interneurons in the control of 5-HT activity. The present study examined this hypothesis using *in vivo* (intracerebral microdialysis) and *in vitro* (immunohistochemistry coupled to reverse transcription-polymerase chain reaction) experimental approaches in rats. Intraperitoneal (0.16 mg/kg) or intra-DRN (1 μM) administration of the selective 5-HT$_{2B}$R antagonist RS 127445 increased 5-HT outflow in both the DRN and the mPFC, these effects being prevented by the intra-DRN perfusion of the GABA$_A$ antagonist bicuculline (100 μM), as well as by the subcutaneous (0.16 mg/kg) or the intra-DRN (0.1 μM) administration of the selective 5-HT$_{1A}$R antagonist WAY 100635. The increase in DRN 5-HT outflow induced by the intra-DRN administration of the selective 5-HT reuptake inhibitor citalopram (0.1 μM) was potentiated by the intra-DRN administration (0.5 μM) of RS 127445 only in the absence of bicuculline perfusion. Finally, *in vitro* experiments revealed the presence of the 5-HT$_{2B}$R mRNA on DRN GABAergic interneurons. Altogether, these results show that, in the rat DRN, 5-HT$_{2B}$Rs are located on GABAergic interneurons, and exert a tonic inhibitory control on 5-HT neurons innervating the mPFC.

1. Introduction

The central serotonin$_{2B}$ receptor (5-HT$_{2B}$R), in keeping with its ability to modulate the activity of the mesocorticolimbic dopamine (DA) pathways, is currently considered as a promising pharmacological target for improved treatments of DA-related neuropsychiatric disorders, such as drug addiction or schizophrenia (Devroye et al., 2018). Recent electrophysiological and microdialysis studies in rats have shown that the opposite effect of 5-HT$_{2B}$R antagonists on accumbal and medial prefrontal cortex (mPFC) DA outflow results from a primary action at the level of the dorsal raphe nucleus (DRN), where they activate 5-HT neurons innervating the mPFC (Devroye et al., 2017). Indeed, the peripheral administration of the selective 5-HT$_{2B}$R antagonist, RS 127445, increases DRN 5-HT neuronal firing and 5-HT release in the mPFC, this latter effect being also observed following its intra-DRN administration (Devroye et al., 2017).

The mechanisms whereby 5-HT$_{2B}$Rs regulate the activity of 5-HT neurons in the DRN remain unknown to date. Interestingly, electrophysiological and neurochemical studies assessing the role of DRN postsynaptic 5-HTRs in the control of 5-HT neurons (Adell et al., 2002; Liu et al., 2000; Quérée et al., 2009; Sharp et al., 2007) raise the possibility that the facilitatory effect of 5-HT$_{2B}$R antagonists on 5-HT neuron activity involves an action on DRN GABAergic interneurons. Indeed, in addition to the self-inhibitory control exerted by somatodendritic 5-HT$_{1A}$ autoreceptors, DRN 5-HT neurons are regulated by a negative-feedback loop involving GABAergic interneurons (Adell et al.,

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2002; Liu et al., 2000; Sharp et al., 2007), which provide a $GABA_A$R-mediated tonic inhibitory input to 5-HT neurons (Celada et al., 2001; Gao et al., 1993; Liu et al., 2000; Tao et al., 1996). Also, GABAergic interneuron activity undergoes excitatory and inhibitory controls exerted respectively by 5-$HT_{2A/2C}$Rs and 5-HT_{1A}Rs expressed by these interneurons (Adell et al., 2002; Day et al., 2004; Liu et al., 2000; Monti, 2010; Serrats et al., 2005; Sharp et al., 2007). These observations raise the possibility that 5-HT_{2B}Rs, as the other members of the 5-HT_2R family, are located on DRN GABA interneurons. This would provide an additional fine-tuning local control of 5-HT activity, such as that observed for 5-HT_{1A}Rs (Liu et al., 2000). In agreement with this view, 5-HT_{2B}R blockade would then reduce a $GABA_A$R-mediated inhibition of DRN 5-HT neurons and enhance their firing activity and forebrain 5-HT release, as previously observed (Devroye et al., 2017).

The present study examined this hypothesis, using *in vivo* intracerebral microdialysis in rats, by studying the role of DRN GABAergic transmission in the effect of RS 127445 on 5-HT outflow in the DRN and the mPFC. A series of experiments involving local and systemic drug administration have been conducted in order to examine the local and distal modulation of 5-HT outflow by RS 127445, and its interaction with other DRN 5-HTRs known to control 5-HT neuron activity. Furthermore, immunohistochemistry coupled to reverse transcription-polymerase chain reaction (RT-PCR) experiments were performed to assess the presence of the 5-HT_{2B}R mRNA on DRN GABAergic interneurons.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (IFFA CREDO, Lyon, France) weighing 280–350 g were used. Animals, housed in collective plastic cages were kept at constant room temperature (21 ± 2 °C) and relative humidity (60%) with a 12 h light/dark cycle (dark from 20:00 h), and had free access to water and food. Animals were acclimated to the housing conditions for at least one week prior to the start of the experiments. All experiments were conducted during the light phase of the light-dark cycle. Animal use procedures were approved by the local ethical committee of the University of Bordeaux (experimental protocol number 50120190-A and A11356) and conformed to the International European Ethical Standards (2010/63/EU) 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.

2.2. Drugs

The following compounds were used: the 5-HT_{2B}R antagonist RS 127445.HCl (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine hydrochloride), the $GABA_A$R antagonist (−)-bicuculline ([R-(R*,S*)]-5-(6,8-Dihydro-8-oxofuro[3,4-e]-1,3-benzodioxol-6-yl)-5,6,7,8-tetrahydro-6,6-dimethyl-1,3-dioxolo[4,5-*g*]isoquinolinium iodide), the 5-HT_{2A}R antagonist MDL 100907 (*R*)-(+)-α-(2,3-Dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperinemethanol, the 5-HT_{2C}R antagonist SB 242084.2HCl (6-chloro-5-methyl-1-[6-(2-methylpiridin-3-yloxy) pyridin-3-yl carbamoyl]indoline.dihydrochloride), the 5-HT_{1A}R antagonist WAY 100635.$C_4H_4O_4$ (*N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate), and the selective 5-HT reuptake inhibitor (SSRI) (1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile hydrobromide), purchased from R&D Systems (Abingdon, UK); the non-steroidal anti-inflammatory drug Meloxicam (METACAM® 2 mg/ml), and the local anesthetic lidocaine (Lurocaine® 20 mg/ml) purchased from Centravet (Dinan, France). All other chemicals and reagents were the purest commercially available (VWR, Strasbourg, France; Sigma-Aldrich, Saint-Quentin Fallavier, France).

2.3. Pharmacological treatments

Bicuculline was first dissolved in distilled water to obtain a 1 mM concentration and then further diluted to the required concentration (100 μM) with artificial cerebrospinal fluid (aCFS) just before its intra-DRN administration by reverse dialysis. RS 127445 was dissolved in a 0.3% Tween 80 distilled water solution, and administered intraperitoneally (i.p.) at 0.16 mg/kg in a volume of 1 ml/kg; WAY 100635 was dissolved in distilled water and administered subcutaneously (s.c.) at 0.16 mg/kg in a volume of 1 ml/kg. When administered locally into the DRN by reverse dialysis, both compounds were first dissolved in distilled water to obtain a 500 mM concentration, and then further diluted to the required concentration (RS 127445: 0.5 or 1 μM; WAY 100635: 0.1 μM) with aCSF just before use. Citalopram was first dissolved in distilled water to obtain a 10 mM concentration, and then further diluted to the required concentration (0.1 μM) with aCSF just before its local administration into the DRN by reverse dialysis. SB 242084 and MDL 100907 were first dissolved in distilled water to obtain a 1 mM concentration, and then further diluted to the required concentration (0.1 or 1 μM) with aCSF just before their intra-DRN administration by reverse dialysis.

Doses, concentrations and pretreatment administration time were chosen according to the pharmacodynamic properties of each drug (Andrews and Johnston, 1979; Bonhaus et al., 1999; Fletcher et al., 1996; Kennett et al., 1997; Kramer et al., 2010; Sur et al., 1998) and on the basis of previous studies reporting its selectivity towards the targeted site (Assié et al., 2005; Auclair et al., 2010; Cunningham et al., 2012; Devroye et al., 2018; Ichikawa and Meltzer, 2000; Müller et al., 2007; Navailles et al., 2008; Tao et al., 2000; Tao and Auerbach, 2002).

All drug doses were calculated as the free base. In each experimental group, animals received either drugs or their appropriate vehicle, according to a randomized design.

2.4. Microdialysis and 5-HT assay

Surgery and perfusion procedures were performed as previously described (Devroye et al., 2017) with minor modifications. Microdialysis probes (CMA/11, cuprophan, 240 μm outer diameter, Carnegie Medicin, Phymep, France) were 4 mm length for the mPFC and 1 mm length for the DRN. Stereotaxic coordinates were chosen according to the atlas of Paxinos and Watson (2005). Rats were anesthetized with 3% isoflurane, and placed in a stereotaxic frame. Two microdialysis probes were simultaneously implanted in the right mPFC (coordinates, in mm, relative to the interaural point: AP = 11.7, L = 0.5, V = 3.7) and in the DRN (20° lateral from vertical, AP = 1, L = −1.5, V = 4.1). After the surgery, the percentage of isoflurane was adjusted to 1.5% until the end of the experiment. Probes were perfused at a constant flow rate (0.5 μl/min), by means of a microperfusion pump (CMA 111, Carnegie Medicin, Phymep), with aCSF containing (in mM): 147 NaCl, 4 KCl, 2.2 $CaCl_2$, pH 7.4.

Pharmacological treatments (see Section 2.3 for details) were performed 120 min after the beginning of the perfusion (stabilization period). 5-HT outflow was monitored during 120 min after the last drug injection. Dialysates were collected in a refrigerated fraction collector (MAB 85 Microbiotech, Phymep) every 20 min.

At the end of each experiment, the animal was deeply anesthetized with a pentobarbital overdose (100 mg/kg, CEVA, Libourne, France), and its brain was removed and fixed in NaCl (0.9%)/paraformaldehyde solution (10%). Probe or injection cannula location into the targeted region was determined histologically on serial coronal sections (60 μm) stained with cresyl violet, and only data obtained from rats with correctly implanted probes were included in the results.

After collection, dialysate samples were immediately analyzed with a high-performance liquid chromatography apparatus (Alexys UHPLC/ECD Neurotransmitter Analyzer, Antec, The Netherlands), equipped with an autosampler (AS 110 UHPLC cool 6-PV, Antec), as previously