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Research Paper

Buspirone requires the intact nigrostriatal pathway to reduce the activity of the subthalamic nucleus via 5-HT_{1A} receptors

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ABSTRACT

The most effective treatment for Parkinson's disease (PD), L-DOPA, induces dyskinesia after prolonged use. We have previously shown that in 6-hydroxydopamine (6-OHDA) lesioned rats rendered dyskinetic by prolonged L-DOPA administration, lesion of the subthalamic nucleus (STN) reduces not only dyskinesias but also buspirone antidyskinetic effect. This study examined the effect of buspirone on STN neuron activity. Cell-attached recordings in parasagittal slices from naïve rats showed that whilst serotonin excited the majority of STN neurons, buspirone showed an inhibitory main effect but only in 27% of the studied cells which was prevented by the 5-HT_{1A} receptor selective antagonist WAY-100635. Conversely, single-unit extracellular recordings were performed *in vivo* on STN neurons from four different groups, i.e., control, chronically treated with L-DOPA, 6-OHDA lesioned and lesioned treated with L-DOPA (dyskinetic) rats. In control animals, systemic-buspirone administration decreased the firing rate in a dose-dependent manner in every cell studied. This effect, prevented by WAY-100635, was absent in 6-OHDA lesioned rats and was not modified by prolonged L-DOPA administration. Altogether, buspirone *in vivo* reduces consistently the firing rate of the STN neurons through 5-HT_{1A} receptors whereas *ex vivo* buspirone seems to affect only a small population of STN neurons. Furthermore, the lack of effect of buspirone in 6-OHDA lesioned rats, suggests the requirement of not only the activation of 5-HT_{1A} receptors but also an intact nigrostriatal pathway for buspirone to inhibit the STN activity.

1. Introduction

As a consequence of long-term treatment with L-DOPA, the most effective drug to alleviate motor symptoms in Parkinson's disease (PD), patients develop L-DOPA-induced dyskinesia (LID). This motor disorder can be completely disabling for the patient, sometimes reducing quality of life even more than PD itself. In spite of a large number of clinical trials for finding an efficient therapy to reduce LID, the only currently available antidyskinetic drug so far, is the anti-influenza agent amantadine (Fox, 2013). Unfortunately, prescription of amantadine raises some controversy about its long-term antidyskinetic efficacy (Thomas et al., 2004; Wolf et al., 2010).

Serotonin (5-HT)-based therapies have been extensively investigated in an attempt to find efficacious antidyskinetic drugs. Indeed, numerous 5-HT agonists have been shown to improve dyskinesia, although not always without worsening the antiparkinsonian effect (Bibbiani et al., 2001; Dupre et al., 2007; Kleedorfer et al., 1991). For example, sarizotan, a 5-HT_{1A} receptor agonist, improves dyskinesia in macaque (Gregoire et al., 2009) and rat models (Gerlach et al., 2011a). However, it failed in a III-phase clinical trial on PD patients (Goetz et al., 2007) due to the fact that sarizotan did not counteract LIDs better than placebo. Also, a single dose of eltoprazine, a 5-$HT_{1A/B}$-receptor agonist, has recently shown antidyskinetic effects in a placebo-controlled phase I/IIa study (Svenningsson et al., 2015) though no data are yet available about its long treatment efficacy.

Abbreviations: 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine; aCSF, artificial cerebrospinal fluid; DRN, dorsal raphe nucleus; LID, L-DOPA-induced dyskinesia; PD, Parkinson's disease; STN, subthalamic nucleus; TH, tyrosine hydroxylase.

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In this regard, buspirone, a partial agonist of 5-HT_{1A} receptors (Peroutka, 1988) has antidyskinetic properties not only in 6-hydroxydopamine (6-OHDA) lesioned rats (Aristieta et al., 2012; Azkona et al., 2014; Dekundy et al., 2007; Eskow et al., 2007; Gerlach et al., 2011b) but also in open-label trials performed in humans (Bonifati et al., 1994; Kleedorfer et al., 1991; Politis et al., 2014). Interestingly, buspirone, apart from being a partial agonist for 5-HT_{1A} heteroreceptors, behaves as a full agonist for 5-HT_{1A}-autoreceptors (Sussman, 1998) in the dorsal raphe nucleus (DRN) inhibiting the serotonergic neuron firing activity (VanderMaelen et al., 1986). Also, it is important to mention that, although buspirone has affinity mainly for 5-HT_{1A} receptors, it also acts as an antagonist for D_2 and D_3 dopamine receptors (Bergman et al., 2013; Dhavalshankh et al., 2007; McMillen et al., 1983). In fact, buspirone reduces graft-induced dyskinesia in a rat model of PD by a mechanism which is independent from activation of either pre- or post-synaptic 5-HT_{1A} receptor (Shin et al., 2012). All these peculiarities could help to explain why buspirone is an efficacious antidyskinetic drug and make it interesting to figure out the target through what buspirone elicits its antidyskinetic effect.

The subthalamic nucleus (STN) is the only glutamatergic nucleus of the basal ganglia and exerts the principal excitatory effect on the output nuclei of the basal ganglia, playing a critical role in the motor circuit. Local infusion of a stimulatory 5-HT_{2C}-receptor agonist into the STN induces orofacial dyskinesia in rat (Eberle-Wang et al., 1996). Moreover, we reported that when the STN is lesioned the antidyskinetic effect of buspirone is decreased (Aristieta et al., 2012). Therefore, the aim of this study was to investigate the effect induced by buspirone on the STN neuronal activity not only in brain slices but also in anaesthetized control and 6-OHDA lesioned rats chronically treated with saline or L-DOPA.

2. Materials and methods

2.1. Animals

Female Sprague–Dawley rats weighing 200–280 g for *in vivo* experiments and male Sprague–Dawley rats weighing 50–75 g for *ex vivo* experiments were housed in groups of six with free access to food and water in environmentally controlled conditions (20 ± 2 °C; 65–70% of relative humidity and a 12 h:12 h light/dark cycle). Every effort was made to minimize animal suffering, to use the minimum number of animals per group and experiment and to utilize alternatives to *in vivo* techniques if available. Experimental procedures were approved by the Local Ethical Committee of the University of Basque Country (UPV/EHU, CEBA/185/2011), following European (2010/63/UE) and Spanish (RD 1201/2005) regulations for the care and use of laboratory animals.

2.2. 6-OHDA lesion and L-DOPA treatment

6-OHDA lesions were performed according to our established protocols (Aristieta et al., 2012; Miguelez et al., 2011). Rats were pretreated 30 min before anesthesia with desipramine (25 mg/kg, i.p.) in order to preserve the noradrenergic terminals. Then, rats were anesthetized with isoflurane inhalation and placed in the stereotaxic frame (David Kopf® Instruments). Lesions were performed by two injections of 6-OHDA of 7.5 μg and 6 μg, respectively (3.5 μg/μl saline with 0.02% ascorbic acid) in the right medial forebrain bundle: 2.5 μl at anteroposterior (AP) −4.4 mm, mediolateral (ML) +1.2 mm and dorsoventral (DV) −7.8 mm, relative to bregma and dura with the toothbar set at −2.4, and 2 μl at AP −4.0 mm, ML +0.8 mm, and DV −8 mm, with toothbar at +3.4 (Paxinos and Watson, 1986). Sham animals were similarly treated but instead of 6-OHDA received vehicle. For the administration of the neurotoxin a 10 μl-Hamilton syringe was used and the infusion rate was 1 μl/min. Prior to the administration, the toxin was kept protected from light and in ice. Two weeks later, the severity of the 6-OHDA lesion was evaluated by rotational behavior over 90 min after d-amphetamine (3 mg/kg, i.p.) administration, and only severely lesioned animals were selected for this study (more than 5 full-body ipsilateral turns/min).

Rats were divided in four different groups depending on the lesion and pharmacological treatment followed: control, sham L-DOPA, 6-OHDA lesioned and 6-OHDA lesioned plus L-DOPA (dyskinetic) rats. Animals were treated chronically over 3 weeks with saline in the control and 6-OHDA lesion groups; or with L-DOPA (6 mg/kg + benserazide-HCl (12 mg/kg s.c.) in sham L-DOPA and the dyskinetic groups. On day 21, the dyskinetic movements were scored as described by Miguelez et al. (2011). All 6-OHDA lesioned animals chronically treated with L-DOPA developed severe dyskinetic movements that reached the peak between 40 and 80 min after a single injection of L-DOPA (Fig. 1A). Tyrosine hydroxylase (TH)-immunostaining was used to examine the degree of dopaminergic denervation in the striatum and the substantia nigra following the methodology described by Miguelez et al. (2011) and in all cases the loss of striatal DA fibers was >90% (Fig. 1B).

2.3. Electrophysiological recordings

2.3.1. Ex vivo recordings

Male Sprague Dawley rats (50–75 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and sacrificed by decapitation. The brain was rapidly dissected out and placed in ice-cold cutting solution containing (in mM): 20 NaCl, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.25 NaH_2PO_4, 85 sucrose, 25 D-glucose and 60 $NaHCO_3$, saturated with 95% O_2/5% CO_2. The cerebellum was removed and brain hemispheres were separated along the inter-hemispheres line. Parasagittal brain slices (300–350 μm thick) containing the STN, identified as a lens-shaped dense patch located medial to the internal capsule, were cut and immediately placed in the artificial cerebrospinal fluid (aCSF) composed of (mM) 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 11.1 D-glucose, 21.4 $NaHCO_3$, 0.1 ascorbic acid, and saturated with 95% O_2/5% CO_2 at 33 °C. After at least one hour resting at 33 °C, the slice was submerged in a slice chamber (0.5 ml) mounted on the microscope stage and superfused (2.5–3 ml/min) with the aCSF bathing solution at 31 °C.

STN neurons were visually identified using an upright microscope with infrared optics (Eclipse E600FN, Nikon®) equipped with a 40× water-immersion objective. Patch electrodes were pulled from borosilicate glass capillaries (World Precision Instruments) on a two-stage puller (PC-10, Narishige) to a pipette resistance of 3–5 MΩ. Recordings were made with an Axopatch 200B amplifier and digitized with a Digidata 1322A (Axon Instruments®). To accurately examine spontaneous firing in STN neurons, pipettes were filled with aCSF and recordings were performed in the minimally invasive cell-attached loose patch configuration (20–30 mΩ seal resistance). Data were low-pass filtered at 5 kHz before being digitized at a rate of 10 kHz. Acquisition and all subsequent analysis were carried out with Clampex 10.4. Firing rate histograms were generated by integrating action potential discharge in 10 s bins (Fig. 1C). 54 cells fired spontaneously and regularly at 13 ± 1 Hz. Only 5 neurons fired in burst mode and 4 cells remained silent. This proportion of regularly firing neurons strongly resembles what has been described in the literature (98%) for *ex vivo* STN cells (Wilson et al., 2004). Also, an increase in the temperature from 31 to 35 °C induced a reversible increase in the average frequency of spontaneous firing rate from 13 ± 4 Hz to 19 ± 7 Hz ($n = 6$; paired t test, $p < 0.05$) (Fig. 1D) as it has been described for this nucleus (Heida et al., 2008). All drugs were applied in the perfusion solution in known concentrations.

2.3.2. In vivo recordings

All STN neuron electrophysiological recordings *in vivo* were performed as described by Morera-Herreras et al. (2011), twenty-four hours after the last saline or L-DOPA injection. Rats were anesthetized with urethane (1.2 g/kg, i.p.) and placed in a stereotaxic frame with its