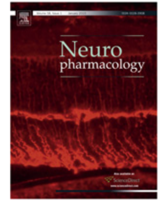
Neuropharmacology 61 (2011) 632–637

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



Activation of 5-HT6 receptors inhibits corticostriatal glutamatergic transmission

Annalisa Tassone [a,b], Graziella Madeo [b], Tommaso Schirinzi [b], Daniela Vita [a], Francesca Puglisi [a,b], Giulia Ponterio [a], Franco Borsini [c], Antonio Pisani [a,b,*], Paola Bonsi [a]

[a] Laboratory of Neurophysiology and Plasticity, Fondazione Santa Lucia I.R.C.C.S., Via del Fosso di Fiorano 64, I-00143 Roma, Italy
[b] Department of Neuroscience, University Tor Vergata, Via Montpellier 1, I-00133 Roma, Italy
[c] Sigma-Tau Industrie Farmaceutiche Riunite SpA, Via Pontina km 30, 400, I-00040 Pomezia, Italy

ARTICLE INFO

Article history:
Received 7 October 2010
Received in revised form
14 April 2011
Accepted 8 May 2011

Keywords:
Serotonin
Striatum
Medium spiny neuron
Cortex
EPSC
Electrophysiology
ST1936

ABSTRACT

We investigated the effect of 5-HT6 receptor subtype activation on glutamatergic transmission by means of whole-cell patch-clamp electrophysiological recordings from medium spiny neurons of the striatum and layer V pyramidal neurons of the prefrontal cortex. To this aim, we took advantage of a novel ligand, ST1936, showing nM affinity and agonist activity at the 5-HT6 receptor subtype. Our data show that 5-HT6 receptor activation by ST1936 reduces the frequency of spontaneous excitatory postsynaptic currents, with an IC50 of 1.3 μM. Moreover, 5-HT6 receptor activation also reduced the amplitude of spontaneous excitatory postsynaptic currents recorded from medium spiny neurons, suggesting a mechanism of action involving postsynaptic 5-HT6 receptors,as further confirmed by the paired-pulse analysis on evoked excitatory postsynaptic currents and by recordings of miniature glutamatergic events. The inhibitory effect of ST1936 on glutamatergic transmission was prevented by the selective 5-HT6 receptor antagonist SB258585 and mimicked by a different agonist, WAY-181187.

Conversely, in the cortex ST1936 reduced the frequency, but not the amplitude, of spontaneous excitatory postsynaptic currents suggesting a presynaptic or indirect effect of the 5-HT6 receptor.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Serotonin (5-HT) exerts diverse physiological and pharmacological effects by acting on multiple receptor subtypes (Tassone et al., 2010; Hannon and Hoyer, 2008). The 5-HT6 receptor is one of the most recent additions to the mammalian 5-HT receptors and has been implicated in psychoemotional, cognitive, and extrapyramidal motor function/dysfunction (Huerta-Rivas et al., 2010; Ohno et al., 2011; for review see: Branchek and Blackburn, 2000; Jones and Blackburn, 2002; Meltzer et al., 2003; Roth et al., 2004; Hannon and Hoyer, 2008; Upton et al., 2008; Tassone et al., 2010). Since 5-HT6 receptor appears to be almost exclusively expressed within the central nervous system, new therapeutic agents targeted on 5-HT6 receptor may have relatively few peripheral side-effects. Indeed, 5-HT6 receptor antagonists have shown beneficial effects in many disorders, which have been ascribed to an enhancing effect on glutamatergic transmission (Dawson et al., 2001; Schechter et al., 2008). However, the absence of selective agonist ligands has severely hampered further investigation on this receptor subtype.

In the present work we directly investigated the effect of a novel 5-HT6 agonist (ST1936; Borsini et al., 2008) on spontaneous glutamatergic transmission by means of whole-cell patch-clamp electrophysiological recordings from both medium spiny neurons (MSNs) of the striatum and layer V pyramidal neurons of the prefrontal cortex (PFC).

Interestingly, our data show that 5-HT6 receptor activation by ST1936 reversibly reduced spontaneous glutamatergic trasmission in both striatal MSNs and layer V PFC pyramidal neurons. The effect of ST1936 was specific on 5-HT6 receptors, as it was prevented by preincubation of the cells in the selective 5-HT6 antagonist SB258585, and was mimicked by the WAY-181187 agonist.

2. Materials and methods

2.1. Corticostriatal slice preparation

Experiments were conducted according to the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the University "Tor Vergata" Ethical Committee. All efforts were made to minimise animal suffering, and to reduce the number of animals used. Three to four-week old male Wistar rats were utilized for the experiments. Animals were sacrificed by cervical dislocation under ether anaesthesia and the brain immediately removed from the skull. As previously

* Corresponding author at: University Tor Vergata, Department of Neuroscience, Via Montpellier1, 00133 Roma, Italy. Tel.: +39 0672596010/06501703153; fax: +39 0672596006/06501703316.
E-mail address: pisani@uniroma2.it (A. Pisani).

0028-3908/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2011.05.004

described (Martella et al., 2005; Sciamanna et al., 2009), coronal slices (200 μm) of prefrontal cortex or striatum were cut with a vibratome in Krebs' solution (in mM: 126 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 10 glucose, 18 $NaHCO_3$), continuously bubbled with 95% O_2 and 5% CO_2. After 30–60 min recovery, individual slices were transferred to a recording chamber (0.5–1 ml volume), superfused with oxygenated Krebs' medium, at 2.5–3 ml/min flow rate (32–33 °C) and placed on the stage of an upright microscope (BX51WI, Olympus) equipped with a water immersion objective (XLUMPlan, Olympus).

2.2. Electrophysiology

Whole-cell patch clamp recordings were performed from either medium spiny neurons (MSNs) or layer V pyramidal neurons visualized using infrared differential interference contrast microscopy in the dorsal striatum or in the prefrontal cortex, respectively (Pisani et al., 2004; Sciamanna et al., 2009). Recordings were made with a Multiclamp 700b amplifier (Axon Instruments), using borosilicate glass pipettes (1.5 mm outer diameter, 0.86 inner diameter) pulled on a P-97 Puller (Sutter Instruments). Pipette resistances ranged from 2.5 to 5 MΩ. Membrane currents were continuously monitored and access resistance measured in voltage-clamp was in the range of 5–30 MΩ prior to electronic compensation (60–80% routinely used). Current–voltage relationships were obtained by applying 50 pA steps in both depolarizing and hyperpolarizing direction (from −500 to 500 pA, 600 ms). For spontaneous excitatory postsynaptic currents (sEPSCs), pipettes were filled with an internal solution containing (in mM): K+-gluconate (125), NaCl (10), $CaCl_2$ (1.0), $MgCl_2$ (2.0), 1,2-bis (2-aminophenoxy) ethane-*N*,*N*,*N*,*N*-tetra-acetic acid (BAPTA) (1), Hepes (10), GTP (0.3) Mg-ATP (2.0); pH adjusted to 7.3 with KOH. Bicuculline (10 μM), or picrotoxin (50 μM), was added to block GABAA-currents. MSNs were clamped at the holding potential (HP) of −80 mV, whereas pyramidal neurons at HP = −70 mV. Tetrodotoxin (TTX, 1 μM) was added to the perfusion solution in order to record miniature EPSCs (mEPSCs) from MSNs. Data were acquired with pCLAMP 9.2 (Axon Instruments) and analyzed off-line with MiniAnalysis 5.1 (Synaptosoft) software. For each recording of sEPSCs and mEPSCs, visual inspection allowed detection of false events. The threshold amplitude for the detection of an event was adjusted above root mean square noise level (<5 pA). Only cells that exhibited <25% changes in frequency during the control samplings were analyzed. A bipolar stimulating electrode was located intrastriatally, close to the recording electrode (50–150 μm), to evoke, in picrotoxin (50 μM), glutamate-mediated synaptic currents (eEPSCs) in MSNs. Stimuli of increasing intensity and duration (5–15 V stimulation range, and 20–40 μs duration range) were delivered at 0.1 Hz. Stimulation strength was adjusted to elicit ∼50% of the maximal eEPSC amplitude, as determined by the input/output relationship. eEPSCs were fully blocked by MK-801 (30 μM) and CNQX (10 μM) (not shown). Baseline current level was detected as mean current value recorded between 10 and 50 ms before electrical stimulation. The maximal current value recorded between 10 and 150 ms after stimulation was considered as eEPSCs peak. eEPSC amplitude was obtained by subtracting the baseline current value to the peak current value. Only cells that had stable baseline and peak-current levels for at least 10 min before drug application were used for statistical analysis. Except for TTX (Alomone Lab), all drugs were from Tocris Cookson (UK) and were applied by switching the control perfusion to drug containing solution. ST1936 was from Sigma–Tau (Pomezia, Italy).

2.3. Statistics

Data are presented as mean ± SEM. Statistical significance of the difference between groups was evaluated using GraphPad Prism 3.02 software with nonparametric matched-pairs Wilcoxon or repeated measures Friedman tests, where appropriate; the significance level was set at $p < 0.05$ (*).

3. Results

Drugs acting at 5-HT6 receptors have been reported to modulate glutamatergic transmission (Dawson et al., 2001; Schechter et al., 2008). In the present work we investigated the effect of a novel agonist showing Ki affinity values ranging between 24 and 49 nM for the rat cloned 5-HT6 receptor subtype (ST1936; Borsini et al., 2008), on corticostriatal glutamatergic transmission in MSNs of the striatum. The effect of 5-HT6 receptor activation was investigated on spontaneous excitatory postsynaptic currents (sEPSC) by means of whole-cell patch-clamp recordings from IR-DIC-visualized striatal MSNs in acute rat brain slices. The electrophysiological properties confirmed the morphological identification of MSNs: these cells showed a hyperpolarized resting membrane potential (RMP) (−78.3 ± 0.9 mV; $n = 25$), a long delay to first spike, a fast inward rectification, and a long depolarizing ramp to spike threshold underlain by potassium currents (Fig. 1A; Calabresi et al., 1987; Rasmussen et al., 2005). Basal spontaneous glutamatergic transmission, recorded from MSNs in the presence of either bicuculline (10 μM) or picrotoxin (50 μM) to block GABAergic transmission, had a mean frequency of 4.0 ± 0.7 Hz and a mean amplitude value of 9.3 ± 1.5 pA (Fig. 1B; $n = 18$; Centonze et al., 2008). The recorded sEPSCs were abolished by perfusion of the slice with the selective NMDA and AMPA receptor antagonists, MK801 (30 μM) and CNQX (10 μM), as expected. The effect of the novel, selective 5-HT6 receptor agonist ST1936 on corticostriatal glutamatergic transmission was investigated by analyzing the effect of increasing doses of the drug on sEPSC frequency and amplitude. Bath-application of ST1936 (100 nM–20 μM) had no detectable effects on membrane properties measured either in voltage-clamp or current-clamp condition (e.g. membrane capacitance and resistance, membrane potential and holding currents). Bath application of ST1936 at 100, 300, and 500 nM (10 min) did not cause significant effects either on frequency or on amplitude of sEPSCs (Fig. 1C; $n = 12$; $p > 0.05$ Wilcoxon test). Increasing the concentration of ST1936 to 1 μM significantly and reversibly reduced the frequency of sEPSC in 10 out of 12 MSNs (Figs. 1B,C and 2A; 72.9 ± 7.0% of control; $n = 12$; $p < 0.01$ t test). The dose-response curve of the inhibitory effect of ST1936 on the frequency of sEPSCs showed an IC50 value of 1.35 ± 0.5 μM (Fig. 1C; $n = 3$–5 for each point). Interestingly, also the amplitude of striatal sEPSCs was reduced by ST1936 perfusion (Fig. 2A; 83.6 ± 6.8% of control; $n = 12$; $p < 0.05$ t test). For comparison, a further set of experiments was performed with an established 5-HT6 receptor agonist, WAY-181187 (West et al., 2009). At 200 nM, this drug caused an inhibitory effect on sEPSCs, which was similar to that described for ST1936 (Fig. 2A). In order to assess the selectivity of the action of ST1936 on 5-HT6 receptor, some striatal slices were pretreated with the 5-HT6 receptor antagonist SB258585 (10 μM; Bonsi et al., 2007). SB258585 per se did not alter cell membrane properties, measured either in voltage-clamp or in current-clamp condition, nor did significantly affect striatal glutamatergic transmission (Fig. 2B; in SB258585: 97.7 ± 7.2% of control frequency, 94.1 ± 5.2% of control amplitude; $n = 7$; SB258585 *vs.* control: $p > 0.05$ Wilcoxon test). When 1 μM ST1936 was added to the perfusion solution containing SB258585, the inhibitory effect of the 5-HT6 receptor agonist on both frequency and amplitude of sEPSCs was fully prevented (Fig. 2B; in ST1936 plus SB258585: 106.5 ± 8.0% of control frequency; 88.9 ± 7.6% of control amplitude; $n = 7$; ST1936 in SB258585 *vs.* SB258585: $p > 0.05$ Wilcoxon test; the means of the three groups were not significantly different: $p > 0.05$ Friedman test). As changes in both frequency and amplitude of sEPSCs might suggest pre- and postsynaptic sites of action of ST1936 in the striatum, to further investigate this issue we performed paired-pulse recordings on evoked EPSCs (eEPSCs). However, as functional 5-HT6 receptors have been recently shown to be expressed on striatal cholinergic interneurons, where they exert an excitatory effect (Bonsi et al., 2007), we performed these experiments in the presence of both the muscarinic antagonist scopolamine (3 μM) and the GABAA antagonist picrotoxin (50 μM). In these experimental conditions, bath application of 1 μM ST1936 reduced the eEPSC amplitude to 72.2 ± 4.2% of control (not shown; $n = 3$; $p < 0.05$). When paired intrastriatal stimuli were delivered (Fig. 3A; S1 and S2, 50 ms interstimulus interval, at 0.1 Hz), bath perfusion with ST1936 (1 μM) caused a similar reduction of both S1 and S2 eEPSC amplitudes, without significant changes in the paired-pulse ratio (S2/S1 eEPSC amplitudes; Fig. 3B; control: 1.61 ± 0.07, ST1936: 1.59 ± 0.14; $n = 3$; $p > 0.05$), suggesting a mainly postsynaptic site of action of ST1936 in the striatum. To further confirm the location of the 5-HT6 receptor-mediated action, miniature EPSCs (mEPSCs) were recorded from MSNs (Fig. 3C). In the presence of picrotoxin (50 μM) plus tetrodotoxin (TTX, 1 μM),

Download English Version:

https://daneshyari.com/en/article/2493862

Download Persian Version:

https://daneshyari.com/article/2493862

Daneshyari.com