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Impaired striatal D2 receptor function leads to enhanced GABA transmission in a mouse model of DYT1 dystonia

Giuseppe Sciamanna^{a,b}, Paola Bonsi^b, Annalisa Tassone^b, Dario Cuomo^{a,b}, Anne Tschertter^{a,b}, Maria Teresa Viscomi^b, Giuseppina Martella^{a,b}, Nutan Sharma^c, Giorgio Bernardi^{a,b}, David G. Standaert^d, and Antonio Pisani^{a,b,*}

Antonio Pisani: pisani@uniroma2.it

^aDepartment of Neuroscience, University “Tor Vergata”, Via Montpellier, 1, 00133 Rome, Italy

^bFondazione Santa Lucia I.R.C.C.S., 00143 Rome, Italy

^cMassachusetts General Hospital, Harvard Medical School, Boston, USA

^dUniversity of Alabama at Birmingham, Birmingham, USA

Abstract

DYT1 dystonia is caused by a deletion in a glutamic acid residue in the C-terminus of the protein torsinA, whose function is still largely unknown. Alterations in GABAergic signaling have been involved in the pathogenesis of dystonia. We recorded GABA- and glutamate-mediated synaptic currents from a striatal slice preparation obtained from a mouse model of DYT1 dystonia. In medium spiny neurons (MSNs) from mice expressing human mutant torsinA (hMT), we observed a significantly higher frequency, but not amplitude, of GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) and miniature currents (mIPSCs), whereas glutamate-dependent spontaneous excitatory synaptic currents (sEPSCs) were normal. No alterations were found in mice overexpressing normal human torsinA (hWT). To identify the possible sources of the increased GABAergic tone, we recorded GABAergic Fast-Spiking (FS) interneurons that exert a feed-forward inhibition on MSNs. However, both sEPSC and sIPSC recorded from hMT FS interneurons were comparable to hWT and non-transgenic (NT) mice. In physiological conditions, dopamine (DA) D2 receptor act presynaptically to reduce striatal GABA release. Of note, application of the D2-like receptor agonist quinpirole failed to reduce the frequency of sIPSCs in MSNs from hMT as compared to hWT and NT mice. Likewise, the inhibitory effect of quinpirole was lost on evoked IPSCs both in MSNs and FS interneurons from hMT mice. Our findings demonstrate a disinhibition of striatal GABAergic synaptic activity, that can be at least partially attributed to a D2 DA receptor dysfunction.

Keywords

Electrophysiology; Dystonia; D2 dopamine receptor; Medium Spiny neurons; Fast-spiking interneuron

Introduction

DYT1 dystonia is a severe form of inherited generalized dystonia, caused by a deletion in the DYT1 gene encoding the protein torsinA (Ozelius et al., 1997). The physiological function of torsinA is unclear, though it has been proposed to perform chaperone-like functions, assist in protein trafficking, membrane fusion and participate in secretory processing (Goodchild et al., 2005; Granata et al., 2007; Hewett et al., 2007). The neurochemical basis for primary dystonia is currently unknown, although abnormalities in striatal dopaminergic signalling have been proposed to play a role in the pathophysiology of this disorder (rev. Breakefield, et al., 2008). A reduction of dopamine (DA) levels was found in the putamen and caudate in one DYT1 patient (Furukawa et al., 2000). A subsequent study revealed no difference in total striatal DA content in three DYT1 brains, but an increased DOPAC/DA ratio, suggestive of an increased DA turnover, along with a trend toward decreased D1 and D2 receptor binding (Augood et al., 2002). In non-manifesting carriers of the DYT1 mutation, a PET study revealed a moderate reduction of striatal D2 receptor binding (Asanuma et al., 2005). Similar results have been reported in patients with focal dystonia (Perlmutter et al., 1997).

Evidence on the role of DA transmission emerged also from genetic mouse models of DYT1 dystonia. In mice overexpressing mutant torsinA (Sharma et al., 2005), basal striatal DA levels and binding of D1 and D2 receptors were unaffected (Balcioglu et al., 2007; Zhao et al., 2008). However, amphetamine-induced DA release was reduced. DA metabolite ratios were found either increased (Zhao et al., 2008), or unchanged (Balcioglu et al., 2007). In this same model, we have identified altered D2 receptor responses in striatal cholinergic interneurons (Pisani et al., 2006). Recently, abnormalities in serotonin, but not DA levels were found in another model of DYT1 dystonia (Grundmann et al., 2007).

GABAergic medium spiny neurons (MSNs) are the principal output neurons and the primary target of the dopaminergic nigrostriatal pathway. Inhibitory synaptic inputs to MSNs derive both from axon collaterals of other MSNs, and from GABAergic interneurons, primarily Fast-Spiking (FS), parvalbumin-immunoreactive interneurons (Tepper et al., 2004). Both synthesis and release of GABA are tonically inhibited by D2 receptors (Girault et al., 1986; Delgado et al., 2000). The cellular localization of DA receptors has been extensively studied, showing a predominant expression of D2 receptors on enkephalinergic striatopallidal MSNs (Gerfen et al., 1990). While it has been demonstrated that cholinergic interneurons express D2 receptors (Le Moine et al., 1990), the precise localization of D2 receptors on GABAergic interneurons remains to be established. In particular, it is unclear if GABAergic axon terminals expressing D2 receptors derive from collaterals of other MSNs or from interneurons. The critical role of such interaction led us to hypothesize that mutant torsinA might disrupt dopaminergic regulation of striatal GABA. We found an abnormally increased GABAergic synaptic activity in mice overexpressing the mutant torsinA (hMT), compared to wild-type littermates expressing normal torsinA (hWT). More importantly, D2 receptor activation failed to reduce GABA currents. These findings provide further evidence for a role of D2 receptors in the pathogenesis of dystonia.

Materials and methods

Corticostriatal slice preparation

Experiments were conducted according to the EC guidelines (86/609/EEC) and approved by the University "Tor Vergata". Mice (8-10 weeks old) were generated as described (Sharma et al., 2005). For each strain, non-transgenic littermates were utilized as controls, and are defined as non-transgenics (NT) (C57BL-6). Thus, the genetic background was identical between the control and experimental animals, with the only difference being the

presence of the transgene. Thus, “hWT” indicates mice expressing normal human torsinA, whereas “hMT” mice are those expressing human mutant torsinA. Mice were sacrificed by cervical dislocation under ether anaesthesia and the brain immediately removed from the skull. Coronal corticostriatal slices (200 μm) 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 $^\circ\text{C}$) and placed on the stage of an upright microscope (BX51WI, Olympus) equipped with a water immersion objective (XLUMPlan, Olympus).

Electrophysiology

Whole-cell patch clamping were performed from medium spiny neurons (MSNs) and Fast-Spiking (FS) interneurons, visualized using IR-DIC video microscopy in the dorsal striatum, as described (Kitada et al., 2007; Bonsi et al., 2007). 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 M Ω . Membrane currents were continuously monitored and access resistance measured in voltage-clamp was in the range of 5–30 M Ω 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) was added to block GABA_A -currents. Cells were clamped at the holding potential (HP) of -80 mV.

Spontaneous (sIPSCs) and miniature (mIPSCs) GABA_A -mediated inhibitory postsynaptic currents were recorded at the HP of -75 mV in MK-801 (30 μM) and CNQX (10 μM) to block NMDA and AMPA-mediated glutamate transmission. Pipettes contained (mM): CsCl (110), K^+ -gluconate (30), EGTA (1.1), Hepes (10), CaCl_2 (0.1), Mg-ATP (2.0), GTP (0.3). mIPSCs were recorded in 1 μM Tetrodotoxin (TTX). Under this condition, and according to the equilibrium potential for chloride ions, the synaptic events appeared as downward deflections. Data were acquired with pCLAMP 9.2 (Axon Instruments) and analyzed off-line with MiniAnalysis 5.1 (Synaptosoft) software. For each experiment, 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 $<20\%$ changes in frequency during the control samplings were analyzed.

Bipolar stimulating electrodes were located intrastriatally to evoke, in MK-801 (30 μM) and CNQX (10 μM), GABA_A -mediated synaptic currents (eIPSCs) that were fully blocked by bicuculline (10 μM , not shown). Cells were clamped at 0 mV (HP) and, under such conditions, eIPSCs appear as outward deflections from the baseline (Fig. 8; Centonze et al., 2003). Conversely, at HP of -80 mV, these GABA_A -synaptic currents were detected as inward currents of small amplitude (~ 20 –40 pA). Therefore, in this study, all eIPSCs were recorded at 0 mV. The amplitude of sIPSCs recorded from both MSNs and FS interneurons depended on the stimulation intensity and the distance between the stimulating and the recording electrode (100 ± 50 μm).

Stimuli of increasing intensity and duration (5–15 V stimulation range, and a 20–40 μs duration range) were delivered at 0.1 Hz. Stimulation strength was adjusted to elicit 30–50%

of the maximal eIPSC amplitude, as determined by input/output relationship which was obtained early during recording. eIPSC were analyzed off-line (pClampfit 9.2 software).

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 eIPSCs peak. eIPSC amplitude was obtained 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.

All drugs, but TTX (Alomone Lab) were from Tocris Cookson (UK) and were applied by switching the control perfusion to drugcontaining solution.

Morphology

Randomly, pipettes were loaded also with 2% biocytin to examine the morphology of the recorded neurons. Biocytin-loaded slices were fixed in 4% paraformaldehyde in 0.1 M PB overnight at +4 °C. Slices were then immersed in 30% sucrose and cut into 35 µm-thick transverse sections by a freezing microtome. Sections were immediately incubated with Cy3-conjugated Streptavidin (1:200; Jackson ImmunoResearch Laboratories) in PB 0.3% Triton X-100 for 1h at RT. After 3 washes, sections were incubated with rabbit anti-DARPP-32 (1:200; Millipore) primary antibody in PB 0.3% Triton X-100 overnight at +4 °C. After 3 washes in PB, sections were incubated with a secondary antibody Cy2-conjugated donkey anti-rabbit IgG (1:200; Jackson ImmunoResearch Laboratories). After 3 washes in PB, sections were mounted on slides, air-dried and coverslipped with GEL MOUNT (Biomed). Double fluorescence was examined under a confocal scanning laser microscope (Leica SP5). Final images were projections of z-stack series taken through 20 µm of tissue sections.

Statistics

For data presented as mean±SEM, statistical significance between two groups was evaluated using a paired Student *t*-test, unpaired Student *t*-test or Wilcoxon rank-sum test. Multiple comparisons were analyzed by one-way analysis of variance(ANOVA) followed by post-hoc Tukey's test ($\alpha=0.01$) (Graph Pad Prism 3.02). The significance level was set at $p<0.05$. Kolmogorov–Smirnov test was used to determine differences between two cumulative distributions. One to six neurons per animal were recorded, each electrophysiological measure in the three groups of mice was obtained by pooling data from at least six distinct animals. No more than two cells per animal were included in the statistical analysis of each experimental group; one cell per slice was used for recordings with quinpirole, one to two cells per slice for mIPSCs.

Results

Intrinsic membrane properties of striatal neuronal subtypes

Whole-cell recordings were made of MSNs from the striatum of NT ($n=85$), hWT ($n=81$) and hMT ($n=92$) mice, identified by means of electrophysiological criteria (Kita et al., 1984). MSNs from either NT or hWT and hMT mice did not exhibit firing activity at rest and, upon depolarizing current pulses showed membrane rectification and tonic action potential discharge (Fig. 1). Resting membrane potential (RMP), action potential amplitude, input resistance were similar in the three strains (Fig. 1; Table 1; $p>0.05$) and did not differ from those previously described for mouse MSN (Kitada et al., 2007; Centonze et al. 2003). The current–voltage relationship did not show significant differences among groups (Figs.

1C, D; $p>0.05$). Fig. 1E shows a recorded MSN, double-labelled for biocytin and DARPP-32, showing the peculiar morphological features of a spiny neuron.

Intrinsic membrane properties were used to identify striatal GABAergic FS interneurons and to distinguish them from other striatal neuronal subtypes (Tepper et al., 2004) (Fig. 1; Table 1). Compared to MSNs, FS interneurons ($n=29$) had a more depolarized RMP and higher input resistance. FS interneurons were silent at rest, and upon brief depolarization exhibited early, brief spikes (width at half amplitude <1 ms), and rapidly peaking after hyperpolarizations (Fig. 1, Table 1). In response to depolarizing pulses of longer duration, FS interneurons showed either a brief burst of spikes, or an early spike followed by a tonic firing discharge without accommodation (Fig. 1; Table 1). The membrane properties of FS interneurons did not differ from those measured in hWT ($n=18$) and hMT mice ($n=26$; $p>0.05$).

Spontaneous glutamate- and GABA-dependent synaptic events

The activity of striatal MSNs is driven both by cortical and thalamic glutamatergic inputs. Thus, we measured sEPSCs, which are considered a reliable indicator of glutamatergic activity, from MSNs of NT and transgenic mice. MSNs were clamped at -80 mV and sEPSCs were recorded in the presence of the GABA_A receptor antagonist bicuculline (10 μ M). A combination of NMDA and AMPA glutamate receptor antagonists, MK-801 (30 μ M) and CNQX (10 μ M) fully blocked spontaneous synaptic events (not shown). Most of the sEPSCs recorded from NT MSNs ($n=11$) had amplitude and frequency ranging between 5 and 40 pA, and 0.6 and 7 Hz, respectively and did not differ from hWT ($n=10$) and hMT ($n=11$) MSNs (Fig. S1; $p>0.05$). Kinetic properties, such as rise time, decay time constant and half width, were similar in the three groups (not shown; $p>0.05$).

As an indicator of striatal GABAergic synaptic activity, in a parallel set of experiments we measured inhibitory GABAergic synaptic currents (sIPSCs). Recordings with CsCl-containing pipettes at -75 mV as HP, and in the presence of MK-801 (30 μ M) and CNQX (10 μ M), allowed detection of bicuculline-sensitive sIPSCs (10 μ M). The amplitude (7 – 40 pA) of sIPSCs recorded from NT MSNs (17 ± 2.2 ; $n=15$) was similar to that measured in hWT (20.8 ± 3.2 ; $n=10$) and hMT (21 ± 3 ; $n=11$) mice (Fig. 2; $p>0.05$). The kinetic characteristics of sIPSCs were indistinguishable among the three groups (Fig. 2; $p>0.05$). Conversely, the mean frequency of sIPSCs was significantly increased in hMT mice (3.01 ± 0.5 Hz), compared to both hWT (2.2 ± 0.2 Hz) and NT (Fig. 2; 1.9 ± 0.2 ; $p<0.05$), suggestive of an increased GABAergic input onto MSNs.

To analyze further GABA-dependent synaptic activity, we recorded miniature IPSCs (mIPSCs), in the presence of TTX, to block action potential dependent GABA release. As expected, TTX homogeneously reduced mean frequencies but not amplitudes of synaptic events, if compared to sIPSCs ($\sim 72\%$ of control). However, we found a significantly higher frequency, but not amplitude, of mIPSCs recorded from MSNs of hMT (1.7 ± 0.3 Hz; $n=11$), compared to hWT (1 ± 0.14 Hz; $n=10$) and NT mice (0.99 ± 0.1 Hz; $n=12$) (Fig. 3; $p<0.05$), suggesting that the increase in GABA synaptic events relies, at least partly, upon action potential-independent GABA release.

Spontaneous synaptic events in fast-spiking interneurons

Striatal FS interneurons constitute an interconnected network of GABAergic cells that exert a powerful feed-forward inhibitory control on MSNs (Koós and Tepper, 1999; Tepper et al., 2004). The FS interneurons are, in turn, driven primarily by excitatory afferents from neocortex. To explore the possibility that altered drive to FS interneurons was responsible for the increase in synaptic GABAergic events recorded from MSNs, we measured sEPSCs

from FS interneurons from NT and transgenic mice. Measurement of mean frequency and amplitude of glutamatergic sEPSCs did not reveal significant changes either in hWT (8 ± 2.2 Hz; 8.2 ± 0.9 pA; $n=6$), in hMT (8.8 ± 2.4 Hz; 7.1 ± 0.8 pA; $n=8$) mice, or compared to NT animals (Fig. 4; 9.4 ± 3 Hz; 8 ± 0.8 pA; $n=7$; $p>0.05$). Likewise, no significant difference was measured among sIPSCs (NT= 4.2 ± 0.8 Hz, 15.5 ± 1.5 pA, $n=7$; hWT= 4.3 ± 0.9 Hz; 14.4 ± 2 pA, $n=6$; hMT= 4.2 ± 1.1 Hz; 15.5 ± 2 pA, $n=8$).

Likewise, analysis of kinetic properties for both synaptic events did not differ among the three groups of mice (data not shown, $p>0.05$). Together, these results do not support a major involvement of altered excitatory drive to FS interneurons in the elevation of GABAergic synaptic activity observed in hMT mice.

Effects of D2 DA receptor activation on sIPSCs

In normal conditions, striatal GABA synthesis and release is under the inhibitory control of D2 DA receptors expressed on GABAergic nerve terminals (Delgado et al., 2000; Guzmán et al., 2003; Centonze et al., 2003). To address whether D2 receptor dysfunction was responsible for the increase in sIPSC frequency, we measured the response of MSNs and FS interneurons to the D2-like receptor agonist quinpirole. Although quinpirole can activate both D2 and D3 receptor proteins, the dorsal striatum lacks D3 receptors, and therefore the effects of quinpirole in this region on synaptic currents are presumably mediated by D2 receptors (Le Moine and Bloch, 1996; Guzmán et al., 2003).

In slices from either NT ($n=19$) or hWT mice ($n=17$), bath application of quinpirole (1–30 μ M, 10 min) caused no consistent changes in membrane properties of the recorded cells, and dose dependently reduced the frequency, but not the amplitude of sIPSCs recorded from MSNs (Fig. 5; $p<0.05$). After 10 μ M quinpirole, sIPSC frequency was $83.2 \pm 5\%$ of control (pre-drug) in NT mice, $76.3 \pm 4.2\%$ in hWT mice, whereas amplitude was unaffected (Fig. 5; NT= $97.1 \pm 8\%$; hWT= $86.3 \pm 12\%$, $p>0.05$). The quinpirole effect on sIPSC frequency was prevented by pretreatment with the D2 receptor antagonist α -sulpiride (3 μ M, $98.7 \pm 1.1\%$, and $101.5 \pm 2.2\%$ in NT and hWT, respectively; $n=5$ for both groups; data not shown). On the contrary, 10 μ M quinpirole failed to modify either the frequency ($102.6 \pm 5.3\%$) or the amplitude ($99.2 \pm 6.2\%$) of GABA-dependent sIPSCs recorded from hMT MSNs, as well as at all concentrations tested (Fig. 5B; $n=22$ $p>0.05$). Moreover, quinpirole (10 μ M; 10 min) affected neither frequency or amplitude of mIPSCs in NT ($n=8$) as well as in transgenic mice (Fig. S2; $n=5$ and 7 for both hWT and hMT, respectively; $p>0.05$), a result which is consistent with the notion that mIPSCs are calcium-independent events (Zhu and Lovinger, 2005; Centonze et al., 2008), whereas the D2-dependent inhibition involves calcium channels (Yan et al., 1997). We also tested the possibility of an inhibitory tone mediated by D2 receptors on GABA transmission in NT and hWT mice by bathing the slices with α -sulpiride. Both frequency and amplitude of sIPSCs were not affected by α -sulpiride (3 μ M, 10 min) (NT= 1.7 ± 0.6 Hz; 18.1 ± 3 pA; hWT= 2 ± 0.4 Hz; 19.7 ± 3.3 pA; $n=10$ for each genotype, $p>0.05$; data not shown).

To ascertain if the loss of modulation was specific to D2 receptors or rather it reflects a more generalized alteration of other Gi/Go coupled receptor at GABAergic synapses, we examined the effect of both the GABAB receptor agonist baclofen and the CB1 receptor agonist HU 210 on sIPSCs. HU 210 (1 μ M, 10 min) reduced sIPSC frequency homogeneously in the three strains (Fig. 6; NT= $54.3 \pm 3.1\%$; hWT= $52.1 \pm 3.6\%$; hMT= $57.4 \pm 3.6\%$; $n=5$ for each genotype, $p<0.05$). Conversely, sIPSC amplitude was unaffected (Fig. 6; NT= $102.1 \pm 15.2\%$; hWT= $111 \pm 13.1\%$; hMT= $105.2 \pm 17\%$; $n=5$ per genotype; $p>0.05$). Similarly, 10 μ M baclofen significantly decreased sIPSC frequency (Fig. 6; NT= $74 \pm 5.1\%$; hWT= $75 \pm 6.3\%$; hMT= $69.2 \pm 3.4\%$; $n=5$ per genotype; $p<0.05$), but not the amplitude (Fig. 6; NT= $97.1 \pm 8\%$; hWT= $88.3 \pm 11.2\%$; hMT= $99 \pm 6.4\%$; $n=5$ per genotype;

$p > 0.05$), confirming the specificity of D2 receptor alteration. In addition, to unmask a possible tonic endocannabinoid release, slices were bathed in the presence of a CB1 receptor antagonist, AM251 (10 μ M, 10–15 min). Both frequency and amplitude were unaffected by AM251 in the three strains of mice (frequency: NT: $82 \pm 10\%$; hWT: $84.4 \pm 16.8\%$; hMT: $81.2 \pm 14.4\%$; $p > 0.05$; for amplitude, NT: $94.8 \pm 9.8\%$; hWT: $94.5 \pm 8.9\%$; hMT: $92.3 \pm 7.9\%$; $n = 4$ per genotype, data not shown).

Finally, we analyzed the effect of D2 activation on FS interneurons. No significant change was observed on holding current and firing rate of the recorded interneurons after bath-application of quinpirole. We were unable to detect a modulatory effect of quinpirole (1–10 μ M) on sIPSCs of FS interneurons in slices from NT, hWT or hMT mice (Fig. 7; $n = 7, 6$, and 6 , respectively; $p > 0.05$). After bath application of 10 μ M quinpirole, no significant change was measured (Frequency: $102.4 \pm 3.5\%$ for NT; $100.8 \pm 5.2\%$ for hWT; $99.5 \pm 3.6\%$ for hMT; amplitude: $101.1 \pm 5.1\%$ for NT; $102.6 \pm 4\%$ for hWT; $93.6 \pm 5.9\%$ for hMT; $p > 0.05$).

Absence of D2 DA-dependent inhibition on evoked IPSCs from MSNs and FS interneurons

In another group of experiments, eIPSCs were elicited by intrastriatal electrical stimulation. These synaptic currents were recorded with CsCl-filled pipettes, with cells clamped at 0 mV.

Mean amplitude values, in response to synaptic stimuli able to elicit 30–50% of the maximal eIPSC amplitude (see methods), were 65 ± 15 pA (NT; $n = 8$), 59.5 ± 16.6 pA (hWT; $n = 9$) and 61 ± 17 pA (hMT; $n = 10$).

Bath application of quinpirole (1–30 μ M, 10 min) did not cause any change in RMP or input resistance of the MSNs, but reduced the amplitude of the eIPSC recorded both from NT and hWT mice (Fig. 8; $67.7 \pm 2.1\%$, $66.1 \pm 2.3\%$ of control, respectively at 10 μ M; $n = 12$ for each genotype; $p < 0.05$). This effect was fully prevented by pretreatment with L -sulpiride (3 μ M, not shown). In contrast, in MSNs from hMT mice quinpirole failed to modify the eIPSC amplitude (Fig. 8; $98 \pm 4.6\%$; $n = 12$; $p > 0.05$).

Finally, we examined the effect of quinpirole also on eIPSCs in FS interneurons. Evoked IPSCs were recorded in the same experimental conditions as described for MSNs. The mean amplitudes measured in FS neurons were 47.2 ± 11.5 pA ($n = 14$) for hMT mice, 49.1 ± 12 pA, ($n = 12$) for hWT, and 61.5 ± 13 pA in NT mice ($n = 13$) (Fig. 8). In FS neurons from NT and hWT animals, perfusion of the slice with quinpirole dose-dependently reduced the eIPSC amplitude (Fig. 8; at 10 μ M quinpirole: $68.2 \pm 3.2\%$; $68.3 \pm 3\%$, respectively; $p < 0.05$), without modifying membrane properties. Conversely, quinpirole failed to alter either the amplitude or kinetic properties of eIPSCs in FS interneurons from hMT mice (Fig. 8; 10 μ M quinpirole: $86.9 \pm 1.6\%$; $p > 0.05$).

Discussion

The main finding of the present study is a significant abnormality of striatal GABAergic function in a transgenic mouse model of DYT1 dystonia. These mice do not exhibit overt dystonic symptoms, but display both impairment in motor function and biochemical abnormalities in DA neurotransmission (Sharma et al., 2005; Balcioglu et al., 2007; Zhao et al., 2008). In MSNs from hMT mice, we found an increased GABAergic signaling, measurable in the frequency of both sIPSCs and of mIPSCs. In non-transgenic mice, as well as in the hWT line expressing normal human torsinA, activation of D2 receptors by quinpirole suppresses GABAergic activity. Conversely, in hMT mice, D2 receptor activation fails to inhibit either spontaneous (sIPSC) or electrically stimulated (eIPSCs) GABAergic currents. This finding appears to be selective for D2 receptors, as the inhibitory effects mediated either by GABAB- or CB1 receptors on sIPSC are preserved. These data

demonstrate an increase in GABAergic activity in the striatum in this model of dystonia, and suggest that an impairment of D2 receptor-dependent control may be a major cause.

Enhanced GABAergic synaptic activity in hMT mice

The significant rise in frequency but not amplitude of sIPSCs recorded from MSNs indicate an increased GABA input to projection neurons in hMT mice. In the striatum there are two main types of GABAergic inhibitory circuitry, that might account for such increase. A feed forward inhibitory circuit mediated by no less than three different class of GABAergic interneurons exerting monosynaptic inhibition onto MSNs. Among these, FS interneurons and Low Threshold Spike (LTS) interneurons have been shown to exert powerful control over spike timing in MSNs (Koós and Tepper, 1999). At least one additional type exhibited electrophysiological features distinct from those of FS and LTS (Tepper et al., 2008). The feedback circuit consists of the numerous MSNs providing inhibitory input to neighbouring cells through recurrent axon collaterals. As the number of synapses formed by axon collaterals of MSNs onto other MSNs is significantly greater than that formed by interneurons, one would assume the feedback control to be predominant. However, paired recordings have shown that synaptic activity between MSNs is weaker than that between MSNs-interneurons. Indeed, spiking activity evoked in a FS interneuron elicits reliable GABAergic synaptic currents in MSNs (Koós and Tepper, 1999). Such a difference in synaptic strength arises from a divergent synaptic localization. GABAergic interneurons form their synapses mainly in the somata of MSNs, whereas synapses from recurrent axon collaterals connect MSNs mostly on spines or dendritic shafts (Kita et al., 1990; Guzmán et al., 2003; Tepper et al., 2004). Additionally, FS interneurons are interconnected through gap-junctions, thereby exerting a potent, convergent inhibition on MSNs (Koós and Tepper, 1999). The feedback system mainly controls local dendritic processing, including spike back-propagation and dendritic calcium entry, thereby influencing MSN excitability as well as synaptic plasticity (Plenz, 2003). Notably, it has been recently reported that recurrent collateral synaptic inputs arising from MSNs target differently MSNs expressing D1 or D2 receptors (Taverna et al., 2008). In particular, recurrent synapses arising from D2 MSNs exert a stronger local inhibition than those originating from D1 MSNs (Taverna et al., 2008). Such evidence suggests that the increase in GABA we observed in hMT mice could be explained by the lack of inhibition exerted by MSNs expressing D2 receptors.

We considered FS interneurons as a possible source for the higher GABAergic activity recorded in hMT mice, but found no significant difference both in GABA- and glutamate-mediated currents. This result would suggest to take into account LTS interneurons as another possible source for increased GABA. As reported, at least two different LTS-like cells have been described but a clear differentiation between them has not been determined yet (Tepper et al., 2008). Thus, we did not explore this possibility further in the present work.

Our measurements of mIPSCs offer further insights into the possible mechanisms responsible for the rise in GABAergic inputs to MSNs. Because spontaneous release occurs randomly at both excitatory and inhibitory synapses, independently of action potential generation (Bekkers et al., 1990), our observation that the frequency of these events is higher in hMT mice supports the assumption that, to some extent, the increase in striatal GABA might depend upon an action potential-independent release probably occurring either at MSN-MSN or at FS-MSN synapses.

Loss of D2-dependent inhibition on striatal GABA transmission

Synaptic release is triggered by clustered presynaptic calcium channels. N-type and P/Q-type channels are found at synapses connecting pairs of MSNs and are subject to DA

modulation (Tecuapetla et al., 2007). Yet, nerve terminals from both MSNs and FS interneurons express D2 receptors (Delle Donne et al., 1997), that exert their modulation by coupling to N-type channels (Yan et al., 1997). We have recently described an abnormal coupling of D2 receptors to N-type channels in cholinergic striatal interneurons from hMT mice (Pisani et al., 2006). Consistently, in the present work we show that D2 receptors failed to inhibit both sIPSCs and eIPSCs from MSNs as well as eIPSCs from FS interneurons of hMT mice. The apparent discrepancy in the ability of quinpirole to inhibit spontaneous and evoked synaptic events recorded from FS interneurons can be partially attributed to the different GABAergic innervation when compared to MSNs. While recurrent axon terminals of MSNs express D2 receptors, FS interneurons do not possess a similar recurrent network. Moreover, it remains to be determined if, and to what extent axon terminals of GABAergic interneurons express D2 receptors. Such an important difference in synaptic contacts and receptor distribution may account for the lack of effect of quinpirole on sIPSC recorded from FS interneurons. Conversely, intrastriatal synaptic stimulation might recruit nerve terminals from both MSNs and FS interneurons, explaining the efficacy of quinpirole on evoked IPSCs recorded from both cellular subtypes.

The lack of effect of quinpirole on mIPSCs recorded in the three strains is not surprising, considering that mIPSCs recorded from striatal neurons have been shown to be not only sodium- but also calcium-independent events, whereas quinpirole acts by reducing calcium influx through N-type channels (Yan et al., 1997). Indeed, mIPSCs recorded from MSNs have been shown to be insensitive to the calcium channel blocker cadmium (Centonze et al., 2008). Similarly, both in hippocampal and amygdala neurons CB1 agonists were shown to reduce spontaneous, but not mIPSCs (Hoffman and Lupica, 2000; Zhu and Lovinger, 2005).

These findings support a link between elevation in striatal GABA and loss of D2-dependent inhibition. Accordingly, perturbations of D2 receptor function are invariably associated with an impairment of GABA transmission. In the striatum of D2 receptor knockout mice, the D2-dependent inhibition on GABA currents was lost, and a significant increase in glutamic acid decarboxylase activity was found (Centonze et al., 2003; An et al., 2004). Moreover, DA deafferentation increased both the synthesis and the extracellular levels of striatal GABA (Lindfors, 1993). These similarities should be interpreted with caution, since these might represent adaptive changes caused either by the deafferentation, or by the genetic deletion. An indirect evidence for the role of D2 receptors is also provided by experiments with cocaine and amphetamine, that reduce GABAergic tone via D2 receptors, but through the release of endogenous DA. In 6-hydroxydopamine-treated rats, both these drugs failed to affect GABA currents, although the effect of exogenous quinpirole was preserved (Centonze et al., 2002).

Possible role of acetylcholine in the loss of D2-mediated modulation of GABAergic activity

Cholinergic modulation of striatal GABAergic activity is very complex and occurs through activation both of nicotinic and muscarinic receptors, located pre- and post synaptically, either on MSNs or on interneurons (Pisani et al., 2007). We have shown that cholinergic interneurons from hMT mice respond to D2 receptor activation with a paradoxical, excitatory effect (Pisani et al., 2006). This led us to hypothesize that in these mice endogenous DA, through D2 receptors causes an excessive acetylcholine release. How an enhanced cholinergic tone could determine the loss of the quinpirole-dependent inhibition on GABA activity?

In the striatum, ambient acetylcholine levels regulate endocannabinoid release by activating M1 muscarinic receptors located on MSNs. In turn, endocannabinoids would retrogradely activate presynaptic CB1 receptors to reduce GABA currents (Narushima et al., 2007). Since CB1 receptors are expressed on GABAergic terminals of both MSNs and FS cells

(Narushima et al., 2006, 2007), an excess in acetylcholine levels would massively depolarize MSNs, boosting endocannabinoid-dependent suppression of inhibitory circuits. We ruled out the possibility of an increased tone of endocannabinoids, by using CB1 receptor antagonists, that failed to modify sIPSC frequency and amplitude. Additionally, no differences were found in the responses to CB1 receptor agonists, suggesting no major changes in receptor sensitivity.

D2 receptors have been shown to form heteromeric complexes with nicotinic acetylcholine (nAChRs) receptors, exerting a synergistic action on DA release (Quarta et al., 2007). Because nAChRs rapidly desensitize, it might be postulated that an enhanced cholinergic tone would produce a robust and long-lasting desensitization of nAChRs that could disrupt also D2 receptor function. However, it remains to be established which mechanisms could underlie this phenomenon.

Implications for basal ganglia circuitry and limitations of mouse models

Within the basal ganglia circuitry, a balanced activation of direct and indirect pathways ensures an efficient excitatory centre and a surround inhibition for correct motor control. The indirect pathway should inhibit neuronal populations that are related to unnecessary, unwanted movements. It has been proposed that this “focusing” function of the basal ganglia could be defective in dystonia (Todd and Perlmuter, 1998; Perlmuter and Mink, 2004). Since D2 receptors primarily localize to and inhibit striatopallidal MSNs, a decrease in D2-dependent inhibition of GABA transmission would lead to a loss of inhibition of the GPe (Todd and Perlmuter, 1998; Sohn and Hallett, 2004). Such abnormality in D2 receptor function along with a reduction in D2 receptor binding in clinically unaffected DYT1 gene carriers (Augood et al., 2002) raises the possibility that D2 receptor dysfunction represents a feature of the non-manifesting carrier state, or a dystonic endophenotype.

However, given the complexity of GABAergic striatal microcircuitry, this is a mere inference. Further work is required to define the distribution and function of D2 receptors on GABAergic nerve terminals and, more importantly, to clarify if these D2-expressing terminals derive from interneurons or from recurrent collaterals of other MSNs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig.1.

Characterization of medium spiny neurons (MSNs) and fast-spiking (FS) interneurons. (A) Superimposed sample recordings in current-clamp mode showing voltage responses to both depolarizing and hyperpolarizing current steps (300 pA, 600 ms) in individual MSNs recorded from either hWT (red; RMP = -81 mV) or hMT (black; RMP = -78 mV) mice. Note the long depolarizing ramp to spike threshold, typical of MSNs. (B) Representative traces of FS interneurons in response to current steps (300 pA, 600 ms) in both hyperpolarizing and depolarizing direction in hWT (red; RMP = -74 mV) and hMT (black; RMP = -70 mV) mice. Note the peculiar rate (~100 Hz) and pattern (no accommodation) of spike discharge in these interneurons. C, D. The plots show the current-voltage relationship obtained from MSNs (C) and FS interneurons (D) recorded from the three groups of mice, as indicated. RMP = resting membrane potential. (E) Confocal images of a MSN from the dorsolateral striatum, double-labelled for biocytin and DARPP-32. The arrow identifies the recorded neuron which shows the typical morphological features of MSNs, with spiny dendrites. Scale bar: 10 μ m.

Fig. 2. Changes in GABA-dependent spontaneous inhibitory synaptic currents (sIPSCs). (A) Voltage-clamp recordings from MSNs showing sIPSC (downward deflections) in the presence of MK-801 (30 μ M) and CNQX (10 μ M) in the bathing solution, in slices from NT (a), hWT (b) and hMT (c) mice. Holding potential: -75 mV. (B) The plots show a significant increase in the mean frequency, not amplitude of sIPSCs in hMT mice, as compared to both NT and hWT. (C) The cumulative probability plot of inter-event intervals confirms that hMT mice have a significant higher frequency of sIPSCs ($p < 0.05$, Kolmogorov–Smirnov test), whereas no significant change was found in cumulative amplitude distributions of sIPSCs in the three strains of mice.

Fig. 3. Properties of miniature inhibitory synaptic currents (mIPSCs). (A) Sample recordings of mIPSCs recorded in TTX (1 μ M), MK-801 (30 μ M) and CNQX (10 μ M), in non-transgenic (NT) MSNs (a), hWT (b) and hMT (c) mice. Holding potential: -75 mV. (B) The plots show the increase in the mean frequency of GABAergic mIPSCs, without significant changes in the mean amplitude. (C) Cumulative distribution of inter-event intervals and amplitudes of GABA currents in the three strains of mice.

Fig. 4. GABAergic and glutamatergic spontaneous synaptic events from FS interneurons. (A) Representative recordings of sIPSCs from FS interneurons from NT (a), hWT (b) and hMT (c) mice. Cells were clamped at -70 mV, in the presence of MK-801 (30 μ M) and CNQX (10 μ M). (B) Representative recordings of sEPSCs obtained from FS interneurons from NT (a), hWT (b) and hMT (c) mice. Holding potential=-75 mV, in the presence of bicuculline (10 μ M). A1, B1. Summary plots showing no significant difference both in frequency and amplitude of sIPSCs and sEPSCs, respectively, recorded from the three groups of mice.

Fig. 5.

D2 dopamine receptor activation fails to inhibit sIPSCs in hMT mice. (A) Sample sIPSCs recorded before and 10 min after quinpirole application (10 μ M) in MSNs recorded from NT animals (a,a1), as well as from hWT (b,b1) and hMT mice (c,c1). Cells were clamped at -75 mV, in MK-801 (30 μ M) and CNQX (10 μ M). (B) The plots show the lack of inhibition of quinpirole on sIPSC frequency in hMT mice, compared both to hWT and NT mice. The dose-response curve confirms the lack of efficacy of quinpirole in hMT mice at all the doses tested. Additionally, quinpirole does not affect sIPSC amplitude in the three groups. (C) Cumulative distribution of inter-event intervals and amplitudes in the presence of 10 μ M quinpirole. Asterisk= $p < 0.05$.

Fig. 6.

CB1 and GABAB receptors preserve their inhibitory action in hMT mice. Representative voltage-clamp recordings showing the ability of the CB1 receptor agonist HU 210 (1 μ M) to suppress sIPSCs from MSNs of NT (a), hWT (b) and hMT (c) mice. (B) The plots show that HU 210 reduces the frequency but not the amplitude of the sIPSCs. Note the lack of significant differences among groups. (C) Similarly, the GABAB receptor agonist baclofen (10 μ M) homogeneously reduces the sIPSC frequency, but not amplitude, in the three strains of mice.

Fig. 7.

FS interneurons are insensitive to the quinpirole-mediated inhibition. A. Sample traces showing the inability of quinpirole (10 μ M) to inhibit sIPSCs recorded from FS interneurons of hMT mice. B. The plots summarize the lack of effect of quinpirole both on frequency and amplitude of sIPSCs and show that no difference was found among the three strains.

Fig. 8.

Effects of quinpirole on evoked synaptic currents from MSNs and FS interneurons. (A) The superimposed traces show GABAergic IPSCs evoked by synaptic stimulation (eIPSCs) before (black trace) and after treatment with quinpirole (10 μ M) (grey trace), in MSNs recorded from NT (a), hWT (b) and hMT (c) mice. Note that in hMT mice, the inhibitory effect of quinpirole was negligible. (B) Sample traces of eIPSCs from a NT FS interneuron (a1). The superimposition shows the quinpirole effect (10 μ M; grey trace) on the eIPSC amplitude. The inhibitory effect of quinpirole is preserved in FS interneurons from hWT mice (b1), but is largely reduced in hMT mice (c1). (C) The plots summarize the synaptic inhibition caused by quinpirole, expressed as % of control amplitude in MSNs (left) and in FS interneurons (right). Asterisks= $p < 0.05$. (D) Dose-response curves for the inhibitory effects of quinpirole on eIPSC recorded from MSNs (left) or FS interneurons (right), showing the loss of quinpirole effect in both cell subtypes from hMT mice.

Table 1
Membrane properties of medium spiny neurons (MSNs) and of Fast-Spiking (FS)
GABAergic interneurons recorded from striatal slices of non-transgenic (NT), wild-type
(hWT) and mutant (hMT) mice

MSN	NT	hWT	hMT
Resting membrane potential (mV)	-77 ± 1.7	-76 ± 1.4	-76 ± 2.5
Input resistance ($M\ \Omega$)	807 ± 4.5	83.1 ± 3.9	81.4 ± 6.3
Firing elicited by depolarizing current	Tonic	Tonic	Tonic
Action potential amplitude (mV)	73 ± 3.4	77 ± 2.2	70 ± 3.6
Width at half amplitude (ms)	1.48 ± 0.1	1.33 ± 0.1	1.45 ± 0.2
FS interneurons	NT	hWT	hMT
Resting membrane potential (mV)	-67 ± 5.1	-71 ± 3.4	-70 ± 0.6
Input resistance ($M\ \Omega$)	83.8 ± 10.8	86.7 ± 11.9	84.8 ± 8.8
Firing elicited by depolarizing current	Tonic/Burst	Tonic/Burst	Tonic/Burst
Action potential amplitude (mV)	71 ± 2.5	70 ± 4.2	68 ± 3.1
Width at half amplitude (ms)	0.7 ± 0.01	0.6 ± 0.01	0.7 ± 0.02