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Localization and function of GABA transporters GAT-1 and GAT-3 in the basal ganglia

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INTRODUCTION

GABA is the main neurotransmitter used in the basal ganglia network, and abnormal transmission at specific GABAergic synapses underlies some of the pathophysiological features of various basal ganglia diseases. A tight regulation of GABA homeostasis is essential to mediate normal basal ganglia function. In this manuscript, we will provide a brief overview of the main characteristics of the different subtypes of GABA transporters in the mammalian CNS, and then discuss some of our recent findings and those from other laboratories about the localization and functions of GABA transporters (GATs) in the basal ganglia. This review does not intend to cover the extensive literature on GATs, but will specifically focus on the distribution and regulatory mechanisms by which these transporters modulate neuronal activity and synaptic transmission in the basal ganglia. Because of the limited amount of data available, this review does not aim at generating integrative concepts about GATs function in the basal ganglia. It is rather focused on the presentation of recent findings that have been gathered about these transporters in specific basal ganglia nuclei, and their potential importance for basal ganglia function and dysfunction. For a more comprehensive account of our current knowledge of GAT function in other brain regions, readers are referred to previous reviews (Borden, 1996; Gadea and Lopez-Colome, 2001; Dalby, 2003; Conti et al., 2004).

GENERAL FEATURES OF GABA TRANSPORTERS

GABA is the main inhibitory neurotransmitter in the mammalian brain. After release from presynaptic terminals, GABA is rapidly removed from the extracellular space by GATs, a regulatory mechanism that terminates inhibitory synaptic transmission (Borden, 1996; Richerson and Wu, 2003). GAT transporter type 1 and 3 (GAT-1 and GAT-3, respectively) are the two main subtypes of GATs responsible for the regulation of extracellular GABA levels in the central nervous system. These transporters are widely expressed in neuronal (mainly GAT-1) and glial (mainly GAT-3) elements throughout the brain, but most data obtained so far relate to their role in the regulation of GABA_{\text{A}} receptor-mediated postsynaptic tonic and phasic inhibition in the hippocampus, cerebral cortex and cerebellum. Taking into consideration the key role of GABAergic transmission within basal ganglia networks, and the importance for these systems to be properly balanced to mediate normal basal ganglia function, we analyzed in detail the localization and function of GAT-1 and GAT-3 in the globus pallidus of normal and Parkinsonian animals, in order to further understand the substrate and possible mechanisms by which GABA transporters may regulate basal ganglia outflow, and may become relevant targets for new therapeutic approaches for the treatment of basal ganglia-related disorders. In this review, we describe the general features of GATs in the basal ganglia, and give a detailed account of recent evidence that GAT-1 and GAT-3 regulation can have a major impact on the firing rate and pattern of basal ganglia neurons through pre- and post-synaptic GABA_{\text{A}}- and GABA_{\text{B}}-receptor-mediated effects.

Keywords: GABA transporter, striatum, globus pallidus, substantia nigra, patch clamp recording

GATs CLONING AND PHARMACOLOGY

To date, four different GATs have been described, GAT-1, GAT-2, GAT-3, and the Betain/GABA transporter type 1 (BGT-1). These transporters are members of a large family of 12-transmembrane spanning Na^{+}/Cl^{-} coupled transporters (for review, see Borden, 1996). GAT-1 was the first GAT to be cloned (Guastella et al., 1990). The GAT-1 protein sequence in rat (Guastella et al., 1990), mouse (Liu et al., 1993), and human (Nelson et al., 1990) displays a high degree of homology and nearly identical pharmacological properties (Borden, 1996). The GAT-2 and GAT-3, cloned by Borden et al. (1992), display a higher degree of amino acid identity between each other (67% identity), and with the fourth GABA transporter, BGT-1 (68 and 65% identity for GAT-2 and GAT-3, respectively) than with GAT-1 (~52% amino acid identity). The amino acid sequence of GAT-3 in human, rat, and mouse is virtually identical with only a few substitutions (for review, see Borden, 1996). In contrast to other transporters, BGT-1, cloned by Yamauchi et al. (1992), utilizes both GABA and betaine as substrates.

GATs exchange GABA for Na^{+} and Cl^{-}. The GABA-transporting function of GATs is particularly dependent on the Na^{+} gradient across the membrane. Although Cl^{-} can significantly enhance the
rate of transport, Cl− alone does not drive GABA uptake in the absence of Na+. The proposed stoichiometry for GAT-1, GAT-2, and GAT-3 is 2 Na+:1 Cl−:1 GABA (Loo et al., 2000; Sacher et al., 2002; Karakossian et al., 2005).

GAT-1 can be pharmacologically isolated from GAT-2, GAT-3, and BGT-1. Various drugs have been identified as highly specific GAT-1 inhibitors (for instance, CI966, SKF 89976A, NO-711, and Tiagabine), while SNAP 5114 is a semiselective blocker of GAT-2 and GAT-3, with a higher affinity for GAT-3 than GAT-2 (IC50 ~5 and 20 μM, respectively). However, because GAT-3 is far more abundant in neurons and glia than GAT-2, SNAP 5114 is commonly used as a GAT-3 blocker in studies of GATs regulation of synaptic transmission in the central nervous system. Microdialysis experiments in rodent hippocampus and thalamus have shown that either local or systemic application of GAT-1 antagonists can increase extracellular GABA concentrations by up to 1.5- to 4-folds the basal levels (Richards and Bowery, 1996; Dalby, 2000). Similarly, application of the GAT-2/GAT-3 blocker, SNAP 5114 (100 μM), increases GABA levels in the thalamus by almost 250%, but has no significant effect on hippocampal GABA concentration (Dalby, 2000).

**GATs LOCALIZATION IN THE CNS**

The cellular localization of GABA transporters has been studied in the rat brain using both in situ hybridization for mRNA (Rattray and Priestley, 1993; Brecha and Weigmann, 1994; Augood et al., 1995; Durkin et al., 1995; Jursky and Nelson, 1996; Nishimura et al., 1997; Yasaki et al., 1997; Ficková et al., 1999) and immunocytochemistry for transporters protein (Ikegaki et al., 1994; Augood et al., 1995; Minelli et al., 1995; Itojima et al., 1996; Ribak et al., 1996; Conti et al., 1998). The GAT-1 mRNA is expressed throughout the brain, but particularly enriched in the olfactory bulb, basal ganglia, interpeduncular nucleus, cerebellum, and retina (Augood et al., 1995; Durkin et al., 1995; Yasaki et al., 1997). Immunohistochemical studies using antibodies raised against recombinant proteins have shown that GAT-1 is not only expressed in GABAergic neurons, but also in non-GABAergic cells and glia in certain brain regions (for review, see Eulenburg and Gomez, 2010), although their function in these neurons remains poorly understood.

GAT-2 mRNA is weakly expressed throughout the brain, primarily in arachnoid and ependymal cells, and to a much lesser extent, in neurons and astrocytes (Durkin et al., 1995; Conti et al., 1999). GAT-3 mRNA and protein are found predominantly in glial cells (Radian et al., 1990; Ikegaki et al., 1994; Durkin et al., 1995). The strongest GAT-3 expression is found in the glomerular layer of the olfactory bulb, the inner nuclei of the retina, the thalamic paraventricular nucleus, and the globus pallidus (GP; Clark et al., 1992; Ikegaki et al., 1994; Durkin et al., 1995; Minelli et al., 1996). Some of these studies showed that GAT-3 is nearly absent from the neocortex and cerebellar cortex, and very weakly expressed in the hippocampus (Clark et al., 1992; Brecha and Weigmann, 1994; Ikegaki et al., 1994; Durkin et al., 1995), while others provided evidence for significant neocortical expression in rodents (Minelli et al., 1996, 2003; Pow et al., 2005). Finally, low to moderate levels of BGT-1 are expressed in most brain regions (Durkin et al., 1995; Zhou and Ong, 2004).

**GATs REGULATION OF SYNAPTIC TRANSMISSION AND PLASTICITY**

The effects of GAT-1 modulation on synaptic transmission have been most studied in the CNS. A summary of the main effects of GAT blockade on GABA release and postsynaptic currents in various CNS regions is shown in Table 1. GAT-1 inhibitors increase the decay of evoked IPSCs, while not having significant effects on IPSC amplitude in many brain regions (Roepstorff and Lambert, 1992; Thompson and Gähwiler, 1992; Engel et al., 1998; Overstreet and Westbrook, 2003). GAT-1 inhibitors also increase GABA receptor-mediated tonic conductances in cerebellar granule cells (Rossi et al., 2003) as well as in granule cells and pyramidal neurons of the hippocampal dentate gyrus (Nusser and Mody, 2002; Semyanov et al., 2003; Sipla et al., 2007). A recent study also demonstrated that GAT-1 blockade or genetic deletion of GAT-1 specifically impairs long-term potentiation (LTP) induced by theta burst stimulation (Gong et al., 2009) in the CA1 region of mouse hippocampus. While there is compelling evidence that GAT-1 regulates GABAergic transmission in the hippocampus (Thompson and Gähwiler, 1992; Isaacson et al., 1993; Dragger and Heinemann, 1996; Engel et al., 1998; Nusser and Mody, 2002; Overstreet and Westbrook, 2003; Semyanov et al., 2003), cerebral cortex (Keros and Hablitz, 2005; Bragina et al., 2008; Gonzalez-Burgos et al., 2009), and cerebellum (Rossi et al., 2003), much less is known about the functional role of GAT-1 in the basal ganglia (Rossi et al., 2003; Galvan et al., 2005; Kinney, 2005; Kirmse et al., 2009). Despite its widespread and abundant expression in many brain regions (see Borden, 1996; Eulenburg and Gomez, 2010 for reviews), the role of GAT-3-mediated regulation of GABAergic transmission remains poorly understood compared with GAT-1 functions in most CNS regions, except for the cerebral cortex and some basal ganglia nuclei (Table 1).

**GAT-1 AND GAT-3 IN THE BASAL GANGLIA**

The relative importance of GAT-1 and GAT-3 in the normal and pathological functioning of the basal ganglia, and the possibility that their regulation could be used to achieve beneficial therapeutic responses in basal ganglia disorders remain largely unexplored. In the following sections, we describe the current knowledge of the localization and function of GAT-1 and GAT-3 in various basal ganglia nuclei, and critically discuss their potential relevance as targets for drug therapies of basal ganglia disorders, such as Parkinson’s disease.

**STRIATUM**

**GAT-1 AND GAT-3 LOCALIZATION**

Most neurons in the striatum, including medium spiny projection neurons and several interneuron subtypes are GABAergic (Kawaguchi et al., 1990). The maintenance of homeostasis in extracellular levels of GABA and GABAergic transmission is, therefore, critical for normal striatal functions. Although the chemical phenotype of most striatal GAT-1-positive cells remains to be determined, it is clear that a significant proportion of the GABAergic neurons in the striatum (including medium spiny neurons and a large proportion of parvalbumin-positive interneurons), express mRNA and immunoreactivity for GAT-1 (Augood et al., 1995; Durkin et al., 1995; Yasaki et al., 1997; Wang and Ong, 1999).

In contrast, the evidence for striatal GAT-3 expression remains controversial. Some of the existing in situ hybridization studies have reported negative data (Clark et al., 1992; Durkin et al., 1995),
## Table 1: Summary the effects of GABA transporters blockade on extracellular GABA levels and postsynaptic currents in the CNS

<table>
<thead>
<tr>
<th>Effects/regions</th>
<th>Hippocampus</th>
<th>Cerebellum</th>
<th>Cortex</th>
<th>Striatum</th>
<th>GP</th>
<th>SNr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular GABA levels</td>
<td>↑ (Dalby, 2000)</td>
<td>–</td>
<td>–</td>
<td>↑ (Valdeir et al., 1992)</td>
<td>↑ (Galvan et al., 2005)</td>
<td>↑ (Bahena-Trujillo and Arias-Montano, 1999)</td>
</tr>
<tr>
<td>eIPSC decay</td>
<td>↑ (Roepstorff and Lambert, 1992; Roepstorff and Lambert, 1994; Thompson and Gahwiler, 1992; Isaacson et al., 1993; Draguhn and Heinemann, 1996; Engel et al., 1998; Overstreet and Westbrook, 2003)</td>
<td>–</td>
<td>↑ (Keros and Hablitz, 2006; Gonzalez-Burgos et al., 2008)</td>
<td>↑ (Kirmse et al., 2008)</td>
<td>↑ (Jin et al., 2011)</td>
<td>↑ (Jin et al., 2011)**</td>
</tr>
<tr>
<td>eIPSC amplitude</td>
<td>N (Roepstorff and Lambert, 1994; Thompson and Gahwiler, 1992; Isaacson et al., 1993; Draguhn and Heinemann, 1996; Engel et al., 1998; Overstreet and Westbrook, 2003; Lindsly and Frazier, 2010)</td>
<td>↓ (Roepstorff and Lambert, 1992; Overstreet et al., 2000; Safiulina et al., 2009)</td>
<td>↑ (Keros and Hablitz, 2006)</td>
<td>↓ (Kirmse et al., 2008)</td>
<td>N</td>
<td>Jin et al., 2011)</td>
</tr>
<tr>
<td>sIPSC decay</td>
<td>N (Jensen et al., 2003)*</td>
<td>↑ (Chiu et al., 2005)</td>
<td>↑ (Kinney, 2009)**</td>
<td>–</td>
<td>↑ (Chen et al., 2003)</td>
<td>N</td>
</tr>
<tr>
<td>sIPSC amplitude</td>
<td>N (Jensen et al., 2003)*</td>
<td>N (Chi et al., 2006)</td>
<td>↑ (Kinney, 2009)**</td>
<td>–</td>
<td>N</td>
<td>Chen et al., 2003)</td>
</tr>
<tr>
<td>sIPSC frequency</td>
<td>N (Jensen et al., 2003)*</td>
<td>–</td>
<td>↑ (Kinney, 2009)**</td>
<td>–</td>
<td>↓ (Chen et al., 2003)</td>
<td>↑ (Jin et al., 2011)**</td>
</tr>
<tr>
<td>mIPSC decay</td>
<td>N (Overstreet et al., 2000)</td>
<td>–</td>
<td>–</td>
<td>N (Kirmse et al., 2008)</td>
<td>N</td>
<td>Jin et al., 2011)</td>
</tr>
<tr>
<td>mIPSCs amplitude</td>
<td>↓ (Overstreet et al., 2000)</td>
<td>–</td>
<td>–</td>
<td>↓ (Kirmse et al., 2008)</td>
<td>N</td>
<td>Jin et al., 2011)</td>
</tr>
<tr>
<td>mIPSC frequency</td>
<td>N (Jensen et al., 2003)*</td>
<td>–</td>
<td>–</td>
<td>↓ (Kirmse et al., 2008)</td>
<td>N</td>
<td>Jin et al., 2011)</td>
</tr>
<tr>
<td>Tonic currents</td>
<td>↑ (Jensen et al., 2003; Nusser and Mody, 2001; Semyanov et al., 2003; Sipila et al., 2007)</td>
<td>↑ (Chiu et al., 2005)</td>
<td>↑ (Keros and Hablitz, 2005)*</td>
<td>↑ (Kirmse et al., 2008)</td>
<td>↑ (Jin et al., 2011)</td>
<td>↑ (Jin et al., 2011)**</td>
</tr>
<tr>
<td>eEPSC amplitude</td>
<td>–</td>
<td>↓ (Gonzalez-Burgos et al., 2008)</td>
<td>–</td>
<td>–</td>
<td>↓ (Jin et al., 2009)</td>
<td>↓ (Jin et al., 2009)**</td>
</tr>
</tbody>
</table>

No Symbol (GAT 1 blockers) *GAT-1-deficient mice, # monkeys, ** GAT3 blocker, ↑ increase, ↓ decrease, N (no change), – (data not available), + GAT1 plus GAT3 blockers.

Abbreviations: GP, globus pallidus; Snr, substantia nigra pars reticulata; eIPSC, evoked inhibitory postsynaptic current; sIPSC, spontaneous inhibitory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current.
while others using similar methods or polymerase chain reaction (rt-PCR) techniques have demonstrated a significant level of striatal GAT-3 mRNA expression in the rat caudate–putamen (Yasumi et al., 1997; Ficková et al., 1999). GAT-3 immunoreactivity was also demonstrated in the monkey striatum (Ng et al., 2000). The cellular and chemical phenotypes of GAT-3-positive striatal elements remain poorly characterized in both primates and non-primates.

**FUNCTIONAL ROLE OF STRIATAL GAT-1**

Systemic or local application of the selective GAT-1 inhibitor, SKF 89976A, doubles the extracellular concentration of GABA in the rat striatum (Waldmeier et al., 1992). Consistent with this finding, GAT-1 blockade induces GABA_A receptor-mediated tonic inhibition of striatal neurons (Kirmse et al., 2008), similar to previous reports in the hippocampus (Jensen et al., 2003; Semyanov et al., 2003; Scimemi et al., 2005) and cerebellar Purkinje cells (Chiu et al., 2005). Electrophysiologic brain slice recording studies have demonstrated that bath application of the GAT-1 inhibitors NO-711 prolongs the decay time of IPSCs evoked locally in striatum (Kirmse et al., 2008) and decreases the amplitude of eIPSCs produced by intrastriatal stimulation. The latter effect is most likely caused by a presynaptic mechanism because it was associated with a significant increase of the paired-pulse facilitation ratio (PPR; Figures 1A,B).

Interestingly, coapplication of NO-711 and the GABA_A-receptor antagonist CGP55845 only partly restored the GAT-1 blockade-mediated effects on the amplitude of eIPSCs but reduced the PPR to control levels (Figures 1C–E), suggesting that the effects of GAT-1 blockade upon eIPSCs are partially, but not fully mediated by GABA_A receptor-dependent mechanisms (Kirmse et al., 2008). This observation was recently extended to the hippocampus (Safiuлина and Cherubini, 2009; Lindsly and Fraxier, 2010). Other mechanisms, including postsynaptic shunting and GABA_A-receptor desensitization due to a persistent activation of GABA_A receptors by high ambient GABA concentration in the presence of NO-711 could also contribute to these effects (Overstreet et al., 2000; Keros and Hablitz, 2005; Kirmse et al., 2008).

GATs, acting in reverse direction, also contribute to the action potential-independent release of GABA in the rat striatum (Bernath and Zigmond, 1989; Del Arco et al., 1998; Schoffelmeer et al., 2000). For instance, microdialysis studies have shown that nipecotic acid, a non-selective GAT blocker, attenuates the amphetamine-induced increase in extracellular concentration of GABA in the striatum of freely moving rats, using a calcium-free microdialysis medium (Del Arco et al., 1998). Nipecotic acid also attenuates glutamate NMDA and dopamine D1-like receptor-mediated [³H]-GABA release from striatal slice and cultured striatal neurons in the presence of the sodium channel blocker tetrodotoxin (Schoffelmeer et al., 2000). These results demonstrate that the release of GABA induced by amphetamine or activation of D1-like or NMDA receptors involves a GAT mechanism. This reversal of GAT function may play a role in the behaviorally activating effects of psychostimulant drugs (Schoffelmeer et al., 2000).
is possible that these transporters might play complementary and synergetic roles towards the regulation of GABAergic transmission in the striatum.

**GLOBUS PALLIDUS**

**GAT-1 AND GAT-3 LOCALIZATION**

The rat GP expresses strong mRNA for both GAT-1 and GAT-3 (Durkin et al., 1995; Yasaki et al., 1997). Consistent with these findings, our studies have demonstrated strong GAT-1 and GAT-3 immunoreactivity in the rat and monkey GP (Galvan et al., 2005, 2010; Jin et al., 2009, 2011; Figure 3). At the electron microscopic level, GAT-1 is largely expressed in small unmyelinated axons in the rat GP (Figure 3), and in both unmyelinated axons and glial processes in the external and internal segments of the GP (GPe and GPi, respectively) in the monkey (Figure 3). The pattern of GAT-3 immunoreactivity in the rat and monkey pallidum is strikingly different from that of GAT-1, being almost exclusively expressed in glial cell processes which, in some cases (Figure 3) are closely apposed to putative GABA ergic terminals forming symmetric synapses or wrapped around axo-dendritic complexes consisting of numerous unlabelled terminals and dendrites of pallidal neurons (Figure 3). Despite significant alterations in GAT function, there is no significant change in the general localization pattern of GAT-1 and GAT-3 in the GPe and GPi of MPTP- treated Parkinsonian monkeys (Figure 3 and below; Galvan et al., 2010).

**FUNCTIONAL ROLE OF GAT-1 IN THE GLOBUS PALLIDUS**

In normal monkeys, local intrapallidal administration of the GAT-1 antagonist (SKF 89976A) significantly increases the ambient GABA level in GPe, as measured by microdialysis (Figure 4A), and reduces the firing rate of GPe and GPi neurons (Figures 4B1, 4B2; Galvan et al., 2005). We found that the inhibitory effects of GATs blockade on GPi firing are strongly decreased in monkeys rendered Parkinsonians by systemic treatment with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTPP), while the effects on GPe discharge rates remain unaffected (Galvan et al., 2010). In line with our monkey data, systemic administration of tiagabine, a GAT-1 antagonist, increases extracellular levels of GABA by up to threefold in the rat GP (Fink-Jensen et al., 1992). Furthermore, bath application of tiagabine has several significant effects when used in rat brain slice recording experiments: (1) GAT-1 inhibition prolongs the decay time of IPSCs evoked by striatal stimulation, without affecting their amplitude (Figures 5A, 5B). Similar observations have been made in many brain regions, suggesting that it represents a general GAT-1 function in the CNS (Roepstorff and Lambert, 1992; Thompson and Gähwiler, 1992; Engel et al., 1998; Overstreet and Westbrook, 2003; for reviews, see Borden, 1996; Richerson and Wu, 2003). (2) GAT-1 blockade induces GABA<sub>\lambda</sub> receptor-mediated tonic currents in rat GP neurons (Jin et al., 2011), another general role reminiscent of GAT-1-mediated effects in other brain regions (for reviews, see Borden, 1996; Richerson and Wu, 2003; Eulenburg and Gomez, 2010). (3) The effects of GAT-1 blockade on spontaneous and miniature IPSCs in rat GP are controversial. On one hand, application of tiagabine prolongs the decay kinetics and reduces the frequency of spontaneous and miniature IPSCs, in part through activation of presynaptic GABA<sub>\lambda</sub> autoreceptors (Chen and Yung, 2003). However, we found that the frequency and amplitude of spontaneous, but not miniature, IPSCs is increased following GAT-1 blockade (Jin et al., 2011). The sources of the discrepancy between these different sets of data remain to be established. (4) GAT-1 blockade reduces the frequency, but not the amplitude of mEPSCs (Figures 5E, 5G), most likely through GABA<sub>\lambda</sub> receptor-mediated presynaptic inhibitory effects upon glutamatergic transmission (Jin and Smith, 2009).

The behavioral effects of GAT-1 blockade in the GP are poorly characterized. Apart from evidence that the unilateral administration of tiagabine in the rat GP induces ipsilateral rotations in rats (Chen and Yung, 2003), very little is known about the influence of GATs on behavior. Given the prominent role of altered GABAergic transmission in parkinsonism (Galvan and Wichmann, 2007), it would be particularly interesting to examine the potential antiparkinsonian effects of GATs. Taking into consideration the physiological effects of GAT-1 blockade on pallidal activity and the proposed pathophysiology of basal ganglia networks in parkinsonism (DeLong, 1990), one could predict that GAT-1 blockade in GPe could induce or exacerbate parkinsonism, due to the increased GABAergic transmission along the indirect pathway, while GAT-1 blockade in GPi could alleviate parkinsonism through increased inhibition of the overactive basal ganglia pallidal outflow to the thalamus and brainstem (DeLong, 1990). However, because we found that the effects of GAT-1 blockers in GPi of Parkinsonian monkeys are altered from normal (Galvan et al., 2010), these speculations are, at best, incomplete with the available data.
evoked IPSCs after striatal stimulation (Figures 5C,D), increases the frequency and amplitude of spontaneous IPSCs, and induces GABAA receptor-mediated tonic currents in GP neurons (Jin et al., 2011). The mechanisms by which GAT-3, but not GAT-1, blockade increases the amplitude of evoked IPSCs are unknown.

One hypothesis put forward in our recent study (Jin et al., 2011) relates to the fact that GAT-3 blockade may result in the activation of a large pool of striatal GABAergic projections neurons and evoked IPSCs after striatal stimulation (Figures 5C,D), increases the frequency and amplitude of spontaneous IPSCs, and induces GABAA receptor-mediated tonic currents in GP neurons (Jin et al., 2011). The mechanisms by which GAT-3, but not GAT-1, blockade increases the amplitude of evoked IPSCs are unknown.

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GABAergic and glutamatergic transmission in the rat GP (Jin et al., 2009, 2011). These differential effects are consistent with localization studies showing that GAT-3 expression in the striatum is comparatively light, compared with the very strong glial expression in the GP (Figure 3; Clark et al., 1992; Durkin et al., 1995; Yasumi et al., 1997; Ng et al., 2000; Galvan et al., 2005; Jin et al., 2009, 2011). GAT-1 and GAT-3, thus, represent differential target sites through which GABA reuptake may subserve a complementary regulation of GABAergic and glutamatergic transmission in the pallidum.

SUBThALAmIC nUclEUS And SUBSTAnTiA niGrA

Despite their distinct glutamatergic phenotype, rat and human STN neurons, display intense GAT-1 mRNA expression (Yasumi et al., 1997; Augood et al., 1999). There is also evidence from other

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FIGURE 5 | Effects of GAT-1 and GAT-3 blockade on GABAergic and glutamatergic synaptic transmission in the rat GP (A) Application of SKF 89976A increases the decay time, but not the amplitude of IPSCs evoked in GP neurons after striatal stimulation. (B) Bar graph showing that SKF 89976A increases the decay time, but has no effect on the amplitude and baseline holding currents of eIPSCs expressed as percent of control ± SEM (* P < 0.005). (C) Application of SNAP 5114 increases the amplitude and decay time of IPSCs evoked in GP neuron by striatal stimulation. (D) Bar graph summarizing the effects of SNAP 5114 on eIPSCs amplitude, decay time, and holding current expressed as percent of control ± SEM (* P < 0.005). (E,F) Sample traces showing mEPSCs recorded in control condition and during SKF 89976A or SNAP 5114 application. (G,H) The summary bar graphs show that SKF 89976A or SNAP 5114 significantly reduce the frequency, but not amplitude of mEPSCs. * P < 0.01. For more details see Jin et al. (2009, 2011).
brain regions such as cerebral cortex, inferior colliculus, and the deep cerebellar nuclei, that the number of GAT-1 mRNA-containing cells is much larger than that of GABAergic (GAD, mRNA-) positive cells (Swan et al., 1994; Yasaki et al., 1997), suggesting that GAT-1 expression extends beyond GABAergic neurons in these brain regions (Yasaki et al., 1997). The functional significance of GATs in non-GABAergic neurons remains unknown (Augood et al., 1999). However, it is interesting to note that an ongoing gene transfection clinical trial in PD aims at changing the phenotype of subthalamic neurons into GABAergic cells in order to reduce the overactive glutamatergic outflow from the subthalamic nucleus (Lewitt et al., 2011). The expression of GAT-1 into subthalamic terminals may increase the likelihood of success of this approach. As in other basal ganglia nuclei, GAT-3 immunoreactivity is expressed in astrocytic processes throughout the monkey STN (Ng et al., 2000).

The effects of GAT activity on the neuronal activity in the STN are unknown. Because of the preponderance of GABAergic pallidal terminals in the STN, and because of the fact that extrasynaptic GABA-B receptors appear to play important roles in the modulation of burst firing and the pallidolysalamic “pacemaker” system (Plenz and Kitai, 1999; Bevan et al., 2002), a better understanding of the mechanisms by which GATs modulate GABAergic STN activity is clearly warranted (for review, see Bevan et al., 2007).

Both GAT-1 and GAT-3 mRNAs are moderately expressed in the rat substantia nigra as a whole. Surprisingly, GAT-1 expression is stronger in dopaminergic pars compacta (SNc) neurons than in the GABAergic pars reticulata (SNr) cells (Durkin et al., 1995; Yasaki et al., 1997), serving as another possible example of GAT-1-mediated function in non-GABAergic neurons. GAT-3 immunoreactivity is also strongly expressed in the monkey SN, where it appears to be preferentially associated with astrocytes (Ng et al., 2000). GAT-1 inhibition significantly reduces [3H] GABA uptake in synaptosomes prepared from the rat SNr (Bahena-Trujillo and Arias-Montano, 1999), and significantly increases extracellular GABA levels in the rat substantia nigra (Fink-Jensen et al., 1992). However, the overall regulatory functions of GATs on synaptic transmission in the SN remain unexplored.

GATs AND PARKINSON'S DISEASE

The data discussed in this review highlight the fact that GAT-1 and GAT-3 represent different target sites through which GABA reuptake may regulate GABAergic and glutamatergic transmission in the basal ganglia. Although still poorly understood, recent in vitro and in vivo data from our laboratory and others have demonstrated that GAT-1 and GAT-3 are strongly expressed in the GP, and that their blockade significantly impacts the activity of pallidal neurons under normal and Parkinsonian conditions, by increasing GABA levels and subsequent overactivation of GABA_{-} and GABA_{+}-receptors (Galvan et al., 2005, 2010; Jin et al., 2009, 2011).

Our recent data from primate experiments demonstrate that the impact of GAT-1 and GAT-3 blockade upon neuronal activity is reduced in the GP of Parkinsonian animals compared with the normal state, despite the fact that the overall pattern of GAT distribution does not change (Galvan et al., 2010). The possible use of drugs that regulate GAT function to treat patients with Parkinson’s disease remains speculative. The essential need of tightly regulated GABA homeostasis for normal brain functions reduces the likelihood that systemic administration of GAT blockers could be done without the risk of significant side effects, although such an approach has shown some therapeutic benefits in patients with epilepsy and anxiety (Gadea and Lopez-Colome, 2001; Dalby, 2003; Sarup et al., 2003; Conti et al., 2004; Schwartz and Nihalani, 2006).

Recent evidence suggests that the interaction of GATs with other receptor systems may provide an alternative strategy for the development of Parkinson’s disease therapy. For instance, a recent study has demonstrated that adenosine inhibits GAT-1-mediated GABA uptake in the rat GP (Gonzalez et al., 2006). This finding, combined with the fact that A2A-receptor antagonists have significant antiparkinsonian effects (Kanda et al., 2000; Chase et al., 2003), raise the possibility that the antiparkinsonian effects of A2A-receptor antagonists are partly due to presynaptic modulation of GABA release at striatopallidal synapses through disinhibition of GAT-1 function. GABA uptake is also modulated by activation of cannabinoid CB1 receptors in the rat GP (Venderova et al., 2005), providing another mechanism that could be used to regulate the overactive GABAergic striatopallidal transmission in Parkinson’s disease (Romero et al., 2002; Brotchie, 2003). These mechanisms of indirect modulation of GAT activity may represent a more promising therapeutic strategy in the treatment of Parkinson’s disease than use of primary GAT blockers (for review, see Conti et al., 2004).

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GABA transporters in the basal ganglia

Jin et al.

GABA transporters: not just for neurotransmitter transport anymore.


