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Nitric oxide regulates synaptic transmission between spiny projection neurons

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Recurrent axon collaterals are a major means of communication between spiny projection neurons (SPNs) in the striatum and profoundly affect the function of the basal ganglia. However, little is known about the molecular and cellular mechanisms that underlie this communication. We show that intrastral nitric oxide (NO) signaling elevates the expression of the vesicular GABA transporter (VGAT) within recurrent collaterals of SPNs. Downregulation of striatal NO signaling resulted in an attenuation of GABAergic signaling in SPN local collaterals, downregulation of VGAT expression in local processes of SPNs, and impaired motor behavior. PKG1 and cAMP response element-binding protein are involved in the signal transduction that transcriptionally regulates VGAT by NO. These data suggest that transcriptional control of the vesicular GABA transporter by NO regulates GABA transmission and action selection.

Throughout the CNS, the volume transmitter nitric oxide (NO) has been implicated in modulating synaptic function, including transmitter release and plasticity (1, 2). The primary target of NO is soluble guanylyl cyclase (sGC), a heterodimer comprising α and β subunits. Upon activation, sGC generates the intracellular messenger cGMP, which, in turn, regulates downstream targets, including channels, phosphodiesterases, and kinases, to alter synaptic function (3). Although sGC is expressed in many adult brain areas, its expression level and activity are highest in the striatum (4, 5), where it is thought to facilitate NO-mediated transmission and plasticity at corticostriatal synapses (6, 7).

To identify plasticity-related molecules that are regulated by NO signaling in striatal neurons, we generated striatal-specific sGCβ1 knockdown (KD) mice and analyzed the resulting transcriptional alterations in a defined population of spiny projection neurons (SPNs). We demonstrated reduction in the levels of the vesicular GABA transporter (VGAT) and GABA signaling at recurrent axon collaterals of striatal SPNs. Signaling between SPNs was also attenuated by pharmacological inhibition of NO signaling. The transcriptional regulation of VGAT by cGMP involved phosphorylation of cAMP response element-binding protein (CREB) by cGMP-dependent protein kinase G (PKG). Our data indicate that NO signaling modulates neurotransmission at neural circuits by modulating the transcription of the vesicular GABA transporter.

Results

Biochemical and Behavioral Characterization of Striatal-sGCβ1 KD Mice. We generated striatal sGCβ1 KD mice to identify the cellular processes regulated by NO specifically in striatal neurons of the adult brain, while avoiding its roles in glutamate nerve terminals, nonneuronal cells, or during neuronal development. For this siGucy, shRNA against Gucy1b3 (the gene encoding sGCβ1) was inserted into an AAV2-delivery system, and its activity was first evaluated in cortical neuron culture. AAV.siGucy or control AAV (AAV.siLuc) were transduced into 87.1 ± 2.13% and 89.7 ± 1.84% of the neurons in the culture, respectively. AAV.siGucy, but not AAV.siLuc, reduced endogenous sGCβ1 levels by 44 ± 12% (Fig. 1 A and B) and attenuated NO-induced production of cGMP by 95 ± 2% (Fig. 1C). Stereotaxic delivery of the virus into the dorsal striatum of adult mice resulted in specific localization of GFP throughout the entire striatum (Fig. 1D), but not the cortex, indicating that corticostriatal neurons were not targeted by the virus. In striata transduced with either AAV.siGucy or AAV.siLuc, over 90% of the GFP-labeled cells were also positive for the 32-kDa dopamine- and cyclic AMP-regulated phosphoprotein (DARPP-32), suggesting that SPNs are the primary target of these viruses (Fig. S1). Notably, AAV.siGucy delivery resulted in a 37 ± 7% reduction of striatal sGCβ1 level (Fig. 1 E and F). In line with this result, we found that sGC activity was dramatically reduced in sGCβ1 KD mice (Fig. 1 G). Interestingly, cAMP levels were not changed in either cultured neurons transduced with AAV.siGucy or in sGCβ1 KD mice, suggesting that its hydrolysis in the striatum is not regulated by cGMP (Fig. 1 C and G).

Basal motor behavior of sGCβ1 KD mice was evaluated using the open-field test. sGCβ1 KD mice had higher motor activity relative to control (Fig. 1H). The induction of motor behavior in sGC-KD mice was not detected in the first 5 min of the test (sGCβ1 KD vehicle 714 ± 76 cm; control 621 ± 95 cm; P = 0.12) (Fig. 1I) whereas acute administration of the nitric oxide synthase inhibitor L-NO-nitroarginine methyl ester, (l-NAME) to sGCβ1 KD mice attenuated movement (90 ± 20 cm) as previously shown in WT (8, 9), suggesting that extrastral NO is a primary target of these viruses (Fig. S1). Notably, AAV.siGucy delivery resulted in a 37 ± 7% reduction of striatal sGCβ1 level (Fig. 1 E and F). In line with this result, we found that sGC activity was dramatically reduced in sGCβ1 KD mice (Fig. 1 G). Interestingly, cAMP levels were not changed in either cultured neurons transduced with AAV.siGucy or in sGCβ1 KD mice, suggesting that its hydrolysis in the striatum is not regulated by cGMP (Fig. 1 C and G).

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Significance

The function of the basal ganglia relies on striatal spiny projecting neurons (SPNs). Axon collaterals of these GABAergic neurons form connections with other SPNs as a means of a feedback inhibition circuit. The mechanisms that regulate neurotransmission at these local synapses remain poorly understood. In this paper, neurotransmission at SPN collaterals is found to be regulated by the gaseous neurotransmitter nitric oxide, which activates a signal-transduction cascade that transcriptionally regulates vesicular GABA transporter specifically at axon collaterals. These findings illustrate a previously unidentified role for nitric oxide in striatal learning and action selection.

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The authors declare no conflict of interest.

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Biochemical and behavioral impairments in striatal-sGCβ1 KD mice. To this end, we used the newly developed translating ribosome affinity purification (TRAP) system (10, 11) and isolated translated mRNA from SNR of the direct projection pathway (dSPNs), qPCR analysis corroborated a 43% reduction in sGCβ1 mRNA in Drd1-expressing SPNs of sGCβ1 KD mice, confirming that SPNs are the target of the AAV (Fig. 2A). We next compared the expression level of ~14,000 genes from dSPNs of sGCβ1 KD-Drd1-TRAP mice to that from mice injected with the control virus. Of the probe sets, 2.7% were down-regulated in dSPNs of sGCβ1 KD mice and 7.5% were induced, compared with control, but none of these genes are associated with glutamatergic signaling. In contrast, among the genes showing the most prominent change was the vesicular GABA transporter (VGAT, Slc32a1), which was reduced by 38 ± 6% in sGCβ1 KD relative to control (Table S1). Of all annotated synaptic vesicle-related genes, 13.5% were differentially regulated in SNR of sGCβ1 KD, including stx3, stx7, synt12, rab9b, kif5a, kif5c, kif5d, kif5f, kif13a, kif17, stxbp1, ptk2r1, ptk3c1, vamp4, and vamp7 (Table S1). However, none of these genes are functionally related to VGAT, suggesting that the transcriptional regulation of VGAT by NO signaling is selective and not part of a complete reorganization in the synaptic vesicle-related proteins. qPCR analysis corroborated a 43 ± 15% reduction in VGAT-translated mRNA in dSPN of sGCβ1 KD mice (Fig. 2B). NO signaling has been implicated in modulating the function of local synapses of SNR as well as primary projections of dSPNs.

The target of the drug. Taken together, these results suggest that striatal NO signaling regulates motor function.

Striatal VGAT Is Down-Regulated in SNRs of sGCβ1 KD Mice. Accumulating data indicate that striatal NO signaling regulates synaptic plasticity (6, 7). We therefore hypothesized that the behavioral impairments in sGCβ1 KD mice are mediated by altering expression of proteins related to plasticity. To identify the genes regulated by NO signaling, we analyzed the translated mRNA expression profile of SNRs from sGCβ1 KD mice. To this end, we used the newly developed translating ribosome affinity purification (TRAP) system (10, 11) and isolated translated mRNA from SNRs of the direct projection pathway (dSPNs), qPCR analysis corroborated a 43% reduction in sGCβ1 mRNA in Drd1-expressing SNRs of sGCβ1 KD mice, confirming that SNRs are the target of the AAV (Fig. 2A). We next compared the expression level of ~14,000 genes from dSNRs of sGCβ1 KD-Drd1-TRAP mice to that from mice injected with the control virus. Of the probe sets, 2.7% were down-regulated in dSNRs of sGCβ1 KD mice and 7.5% were induced, compared with control, but none of these genes are associated with glutamatergic signaling. In contrast, among the genes showing the most prominent change was the vesicular GABA transporter (VGAT, Slc32a1), which was reduced by 38 ± 6% in sGCβ1 KD relative to control (Table S1). Of all annotated synaptic vesicle-related genes, 13.5% were differentially regulated in SNRs of sGCβ1 KD, including stx3, stx7, synt12, rab9b, kif5a, kif5c, kif5d, kif5f, kif13a, kif17, stxbp1, ptk2r1, ptk3c1, vamp4, and vamp7 (Table S1). However, none of these genes are functionally related to VGAT, suggesting that the transcriptional regulation of VGAT by NO signaling is selective and not part of a complete reorganization in the synaptic vesicle-related proteins. qPCR analysis corroborated a 43 ± 15% reduction in VGAT-translated mRNA in dSNR of sGCβ1 KD mice (Fig. 2B). NO signaling has been implicated in modulating the function of local synapses of SNRs as well as primary projections of dSNRs.

Fig. 1. Biochemical and behavioral impairments in striatal-sGCβ1 KD mice. (A) Representative Western blot images depicting endogenous sGCβ1, transduced GFP, and β-actin levels in cortical culture neurons untreated (n = 3 wells) or transduced with either AAV.sGCβ1GFP (n = 3 wells) or the control virus, AAV.sLuc (n = 3 wells). (B) Bar graph summary of the data presented in A. Bars represent mean sGCβ1 levels normalized to β-actin ± SD. (C) Effect of the nitric oxide donor, diethylamine NONOate (DEA, 10 nM) and a phosphodiesterase inhibitor, pentoxifylline (PXF, 150 μM) on cGMP and cAMP levels in neuron culture (n = 3 for each condition, ***P < 0.001). Bars represent mean sGCβ1 levels normalized to β-actin ± SD. (D) Representative gray-scale immunofluorescent images of GFP localization after intrastriatal injection of AAV.sGCβ1GFP, showing GFP expression in the dorsal striatum (arrow, Left). Magnified images (Middle and Right) show the somatic GFP labeling throughout the striatum. (Scale bars: Left, 2 mm, objective 10x; Middle and Right, 200 μm, objective 40x.) (E) Representative Western blot image of protein level from lysates of the entire striata after bilateral intrastriatal injection of either AAV.sGCβ1GFP or control AAV.sLuc, n = 4 mice per group, for controls see S1 Materials and Methods. (F) Quantification of the data presented in E, indicating a persistent reduction in sGCβ1 level by AAV.sGCβ1GFP (⁎P < 0.05). Bars represent mean densities of sGCβ1 normalized to β-actin ± SD. (G) Quantification of cGMP and cAMP levels in control or sGCβ1 KD (n = 6 mice per group, ⋆⋆⋆P = 0.0004). Bars represent levels normalized to tissue wet weight ± SD. (H) Spontaneous motor behavior was recorded for 60 min after vehicle injection. Traveled distance and rest times are illustrated by the open and closed boxes, respectively (n = 6 mice per group, ⋆⋆⋆P < 0.005). (I) Five-minute motor activity in sGCβ1 KD mice was compared with that of mice injected with the nitric synthase inhibitor l-NG-nitroarginine methyl ester (L-NAME) (n = 6 mice per group, ⋆P < 0.05 vs. untreated control).

Fig. 2. VGAT is down-regulated in local processes of striatal SNRs in sGCβ1 KD mice. (A) Bar graph summary of mRNA levels of sGCβ1 (gucy1b1s), VGAT (Slc32a1), synaptophysin (Synp), and α-synuclein (Snca) from control Drd1-Drd1-TRAP mice and sGCβ1 KD Drd1-Drd1-TRAP mice (n = 3 per group, ⋆⋆P < 0.01 vs. control). Bars represent mean mRNA levels normalized to GAPDH, as percentage of control ± SD. (B) Colocalization of VGAT and DARPP-32 in the striatum and substantia nigra pars reticulata (SNR). Representative images from control (n = 5 mice), showing that VGAT is colocalized with DARPP-32 in the striatum. Note in the magnified images that VGAT is expressed in the soma (adjacent to the nucleus in blue) but more prominently in processes (arrows) of SNRs. In sGCβ1 KD (n = 5 mice), VGAT labeling is mainly reduced in processes but is still visible in somas (arrows). In control mice, SNRs’ principal projections to the SNR express VGAT, and the labeling of VGAT in the principal projection remains unchanged in sGCβ1 KD mice. Scale bar: 20 μm.) (C) Mean pixel values were determined concomitantly for DARPP-32 and VGAT in control (n = 5 mice) and sGCβ1 KD mice (n = 5 mice). Analysis of the ratios indicates that VGAT level is reduced in the striatum of sGCβ1 KD mice (⁎⁎⁎P = 0.0005). Bars represent mean ratios of mean pixel values ± SEM.
to the substantia nigra pars reticulata (SNr) (6, 12). To analyze the cellular localization of VGAT in SPNs of sGCβ1 KD mice, brain sections were costained for VGAT and DARPP-32. In the striatum, VGAT was highly but not completely localized in SPNs (Fig. 2B), with a more prominent localization to neuropil, relative to perikarya, indicating its presence in recurrent axon collaterals. Consistent with the reduction in mRNA level, VGAT-protein labeling was 55 ± 15% in the striatum of sGCβ1 KD relative to control mice (Fig. 2B and C). We next compared the level of VGAT in dSPNs and indirect projection SPNs (iSPNs), by analyzing VGAT and GFP immunolabeling in sGCβ1 KD-Drd1-TRAP mice. In line with the TRAP data, the VGAT level in Drd1-positive cells of sGCβ1 KD mice was reduced by 32 ± 5.3% and in Drd1-negative neurons by 25 ± 8.5%, relative to their amplitude were not accompanied by a significant change in the immunolabeling of VGAT in SPNs. VGAT labeling in interneurons of these mice was unchanged (Fig. S3), suggesting that NO signaling regulates VGAT expression in both dSPNs and iSPNs.

GABA Transmission at Recurrent Collaterals of SPNs Is Impaired in sGCβ1 KD Mice. To evaluate the role of sGC on GABA transmission in SPNs, GABA miniature inhibitory post synaptic currents (mIPSCs) were recorded in SPNs. GABA mIPSC frequency was 25% lower in SPNs taken from sGCβ1 KD mice compared with controls (Fig. 3B and C), suggesting a decrease in the release probability of GABAergic synapses onto SPNs (13). Visual inspection of representative records suggested that the frequency of small-amplitude mIPSCs was selectively decreased in SPNs of sGCβ1 KD mice (Fig. 3A). This finding was confirmed by a quantitative analysis of the amplitude distribution of mIPSCs from equal-length records (Fig. 3D). The distribution analysis revealed a 29 ± 8% reduction in the number of mIPSCs with amplitudes less than 65 pA, but not mIPSCs with amplitudes greater than 65 pA (Fig. S2E). The alterations in frequency and amplitude were not accompanied by a significant change in mIPSC kinetics (Fig. 3 F and G), suggesting that GABAergic synapses were not being redistributed. GABAergic synapses onto SPNs are derived from one of three sources: fast-spiking (FS) interneurons, neuronal nitric oxide synthase (nNOS)-positive interneurons, and collateral SPN synapses (14, 15). FS interneurons make perisomatic synapses that give rise to large-amplitude IPSCs. In contrast, nNOS interneurons and SPN synapse primarily upon dendrites and give rise to small-amplitude IPSCs. Because SPNs far outnumber nNOS interneurons, it seems likely that the drop in the frequency of small-amplitude mIPSCs in sGCβ1 KD mice is attributable to a reduction in GABA release from recurrent collaterals.

To physiologically evaluate the function of VGAT in the efferents of dSPNs of sGCβ1 KD mice, the paired pulse ratio of IPSCs in SNr neurons was studied. Striataligral IPSCs were strongly facilitated and were not altered in sGCβ1 KD mice (Fig. S4), suggesting that GABA transmission at extrastriatal terminals of SPNs was unchanged.

To determine whether NO signaling directly modulates recurrent collateral synapses, recurrent collaterals of the indirect pathway were activated optogenetically while recording from a dSPN, (Fig. 4A and B). The IPSCs evoked by collateral activation were significantly diminished by disruption of NOS signaling with l-NAME (100 μM) (Fig. 4C and D). This result demonstrates that NO signaling potentiates recurrent collateral GABAergic synaptic function, in agreement with the inference drawn from the mIPSC analysis.

PKG1 and CREB Mediate the Transcriptional Regulation of VGAT by NO. To elucidate the signaling pathway involved in the transcriptional regulation of VGAT by NO, the phosphorylation level of phosphoproteins involved in striatal and NO signaling, including DARPP-32, ERK1/2, and CREB (6, 16, 17), was assessed. In the striatum, the phosphorylation level of serine 133-CREB was reduced by 62 ± 19% in sGCβ1 KD mice compared with control mice (Fig. 5 A and B), supporting the idea that CREB plays a role in the transcriptional regulation of VGAT by NO. In contrast to CREB, the phosphorylation levels of three-nine 202/tyrosine 204-ERK1/2, and threonine 34-DARPP-32 of sGCβ1 KD mice were 118 ± 9% and 94 ± 13% of the respective control (Fig. 5A and B). We next studied whether CREB phosphorylation is dependent on cGMP and cGMP-dependent kinase (PKG1) in striatal slices. We found a 150 ± 22% increase in CREB phosphorylation after cGMP bath application (Fig. 5 C and D). PKG1 has an established role in directly phosphorylating CREB, and this activity may depend on nuclear shuttling of PKG1 (18). In line with this result, the effect of cGMP was completely abolished by PKG1 inhibition (106 ± 15% of basal phosphorylation level) (Fig. 5 C and D). To identify the subcellular localization of PKG1 after activation by cGMP, the cytoplasmic and nuclear protein fractions were separated. At basal level, PKG1 was localized to both nuclear and cytoplasmic compartments of striatal cells, and cGMP-induced phosphorylation of CREB by PKG1 was not associated with changes in
Disruption of NO signaling reduces iSPN collateral input onto dSPNs. To study the role of dopamine in the regulation of striatal NO signaling and VGAT levels in SPNs, cGMP levels or VGAT labeling was measured in the striata from 6-hydroxydopamine lesioned mice (Fig. 6A). Dopaminergic denervation resulted in a 21 ± 6% reduction in VGAT labeling from the striatum ipsilateral to the lesion (Fig. 6B and C). The reduction in VGAT level was observed both in the neuropil and somas of SPNs. In contrast to the reduction at local processes of SPNs, the level of VGAT at extrastriatal projections to the globus pallidus and substantia nigra was not affected by the lesion (Fig. S6), indicating that dopamine transmission modulates VGAT levels only at recurrent axon collaterals but not at primary projections of SPNs. Chronic l-dopa treatment increased striatal VGAT labeling by 37 ± 12% whereas discontinuation of l-dopa resulted in a 33 ± 11% reduction in the transporter level. Dopamine depletion also resulted in a 53 ± 5% reduction in cGMP levels in the striatum ipsilateral to the lesion (ipsilateral 2.2 ± 0.5 pmol/mg wet weight; contralateral 4.5 ± 0.7 pmol/mg wet weight) (Fig. 6D). Chronic l-dopa treatment increased cGMP levels by 46 ± 11% (5.4 ± 0.9 pmol/mg wet weight; 3.7 ± 0.5 pmol/mg wet weight) whereas its withdrawal resulted in a 49 ± 14 pmol/mg wet weight loss in cGMP levels (2.0 ± 0.5 pmol/mg wet weight). The levels of vgat were reduced in the nuclei (70 ± 6% of control) and the cytoplasm (74 ± 10% of control) (Fig. S5), supporting the idea that the activity of PKG1 in the nucleus may play a role in the transcriptional regulation by NO.

Finally, to study a direct interaction between CREB and regulatory elements in the VGAT gene, the sequence of the VGAT gene (S.32a1) was analyzed, and a conserved cAMP response element (CRE) was identified in its intron (Fig. S5). To evaluate the functionality of this putative CRE, the binding of VGAT to CREB was studied in vitro. A specific interaction was identified between phospho-CREB and the vgat gene (Fig. S5). Moreover, this interaction was dependent on the CRE-like sequence, suggesting that it is functional. To evaluate a possible interaction between vgat and phospho-CREB in vivo, chromatin from either the striatum or hippocampus was isolated, and levels of the gene bound to either phospho-CREB or RNA polymerase II were assessed using chromatin immunoprecipitation (I.p.) and qPCR. The levels of vgat precipitated by RNA polymerase were 4 ± 1.0% and 1.2 ± 0.2% of the striatal and hippocampal gene inputs, respectively (P = 0.04 between brain areas), and the respective levels precipitated by phospho-CREB were 0.3 ± 0.07% and 0.08 ± 0.01%, respectively (P = 0.02 between brain areas), suggesting that CREB plays a role in the transcriptional regulation of VGAT in striatal neurons. Importantly, in striata of sGC1 K.D mice, the binding of vgat by phospho-CREB was reduced by 56 ± 8.7% (Fig. 5G), indicating that CREB-induced transcriptional regulation of vgat is attenuated in sGC1 K.D mice.

Dopamine Signaling Regulates Striatal cGMP and Collateral VGAT Levels in a Coordinated Manner. Striatal dopamine depletion is associated with impairments in both striatal NO signaling and GABAergic transmission in axon collaterals of SPNs (14, 19). To study the role of dopamine in the regulation of striatal NO signaling and VGAT levels in SPNs, cGMP levels or VGAT labeling was measured in the striata from 6-hydroxydopamine lesioned mice (Fig. 6A). Dopaminergic denervation resulted in a 21 ± 6% reduction in VGAT labeling from the striatum ipsilateral to the lesion (Fig. 6B and C). The reduction in VGAT level was observed both in the neuropil and somas of SPNs. In contrast to the reduction at local processes of SPNs, the level of VGAT at extrastriatal projections to the globus pallidus and substantia nigra was not affected by the lesion (Fig. S6), indicating that dopamine transmission modulates VGAT levels only at recurrent axon collaterals but not at primary projections of SPNs. Chronic l-dopa treatment increased striatal VGAT labeling by 37 ± 12% whereas discontinuation of l-dopa resulted in a 33 ± 11% reduction in the transporter level. Dopamine depletion also resulted in a 53 ± 5% reduction in cGMP levels in the striatum ipsilateral to the lesion (ipsilateral 2.2 ± 0.5 pmol/mg wet weight; contralateral 4.5 ± 0.7 pmol/mg wet weight) (Fig. 6D). Chronic l-dopa treatment increased cGMP levels by 46 ± 11% (5.4 ± 0.9 pmol/mg wet weight; 3.7 ± 0.5 pmol/mg wet weight) whereas its withdrawal resulted in a 49 ± 14 pmol/mg wet weight loss in cGMP levels (2.0 ± 0.5 pmol/mg wet weight).

nuclear or cytoplasmic levels of PKG1 (Fig. 5 E and F), suggesting that the activity of PKG1 in the nucleus is not dependent on its nuclear shuttling. In sGC1 K.D mice, the level of PKG1 was reduced in the nucleus (70 ± 6% of control) and the cytoplasm (74 ± 10% of control) (Fig. S5), supporting the idea that the activity of PKG1 in the nucleus may play a role in the transcriptional regulation by NO.
Coregulation of VGAT and cGMP levels by dopamine. (A) Dopaminergic lesion paradigm. Mice received a unilateral injection of either 6-hydroxydopamine (OHDa) or vehicle to the medial forebrain bundle and daily intraperitoneal injections of benserazide (15 mg/kg) with 6-dopa (6 mg/kg, LD) or saline (sal). (B) VGAT and DARPP-32 immunolabeling in the striatum after dopaminergic lesion. Representative images from ipsilateral (ipsi.) and contralateral (cont.) striatum of a mouse treated with 6-hydroxydopamine and saline. Note in the magnified images that VGAT labeling (shown in yellow in the image from the contralateral) is reduced in the soma of SPNs of the ipsilateral striatum (appears green). (Scale bar: 20 μm.) (C) Bar graph summary of ratios of VGAT and DARPP-32 mean pixel values (n = 5-6 mice per group, seven slices per mouse). Bars represent mean ratios in the ipsilateral striatum as percentage of that in the contralateral ± SD. (D) cGMP levels. Bars represent cGMP level in the ipsilateral striatum normalized to tissue wet weight, as percentage of the contralateral ± SD (n = 4-5 mice per group). (E) For each treatment group, the mean cGMP level is plotted against the mean of VGAT labeling ratio.

Discussion

NO signaling has an established role in mediating plasticity and transmission in excitatory synapses, including a role in striatal plasticity at corticostriatal synapses, where it facilitates the response of SPNs to glutamate (6, 7). We have previously found that the NO-sGC-cGMP pathway regulates striatal glutamate signaling through NMDA/AMPA/mGluR5 receptor activation. To identify the molecular mechanisms underlying the role of NO in SPNs, we have used the novel BacTRAP technology. We demonstrated that a loss of NO signaling from striatal neurons of the adult mouse striatum results in a specific reduction in the translated mRNA level of synaptic vesicle-related genes and an impaired GABAergic transmission at recurrent axon collaterals of striatal SPNs. By using a striatal-specific sGCβ1 KD strategy, we have found that transcriptional regulation of VGAT is a major target of NO-sGC signaling, establishing a mechanism by which this signaling pathway can trigger synaptic transmission at GABAergic local synapses. Importantly, we found that SPNs of the two projection pathways, namely the direct and the indirect pathways, are both targets of striatal NO signaling. sGCβ1 mRNA was reduced in dSPN by a similar level to that of the entire striatal tissue, suggesting that the effect is similar in iSPNs. Furthermore, immunolabeling of VGAT was reduced by a similar ratio in both populations in sGCβ1-KD.

Interestingly, sGCβ1-KD mice manifested induction in motor behavior, highlighting asymmetry in the role of NO on local communication between the projection systems. This result suggests that iSPN neurons are more sensitive to the transcriptional regulation of VGAT by NO than dSPNs. In line with this idea, we and others have previously found that axon collaterals between SPNs of the two projection systems are not symmetrical because iSPNs profoundly innervate and efficiently inhibit dSPNs (14, 20, 21). Together with these studies, our experiments suggest that NO signaling regulates motor behavior and action selection by maintaining the normal function of axon collaterals.

We found that cGMP, sGC, PKG1, and CREB are involved in the signaling pathway that transcriptionally regulates VGAT by NO. PKG1 is localized both to the cytoplasm and to the nucleus, and its nuclear levels are reduced in sGCβ1 KD mice, suggesting that PKG1 directly regulates the transcription of downstream genes by phosphorylating nuclear targets, including transcription factors. This finding is in line with the fact that PKG1 can directly phosphorylate CREB (22). By using acute slices, we found that phosphorylation of CREB by cGMP in striatal neurons depends on PKG1, suggesting that cGMP levels at local striatal synapses is unlikely to mediate the transcriptional regulation by NO signaling. Induction in CREB phosphorylation can induce transcriptional activation through binding to the CRE element in target genes (22). In line with this idea, we found a functional CRE in the VGAT gene and identified a loss in the phosphorylation level of CREB and in its binding to the VGAT gene in sGCβ1 KD mice, indicating that CREB positively regulates their transcription. Whether transcriptional regulation of vesicular neurotransmitter transporters is always coordinated with that of other presynaptic and postsynaptic components is still unknown. We found that, in contrast to VGAT, only a small subset of other presynaptic genes were differentially expressed in SPNs of sGCβ1 KD mice. Previous work in cultured neurons showed that the transcriptional regulation of VGAT and other vesicular neurotransmitters transporters is dissociated from that of other components of the synaptic vesicle (23), and our results indicate that such selective regulation is maintained in the adult mammalian brain.

The loss of response of neurons transduced with AAV-siGucy to the NO donor dDAAPN in NO-sGC-KD mice suggested that NO signaling is impaired in sGCβ1-KD mice. Similar to NO, carbon monoxide (CO) is another gaseous neurotransmitter and a ligand for sGC (24). However, CO affinity to sGC is much lower than that of NO, and cGMP induction by CO is significantly lower relative to NO (25). Furthermore, the expression of heme oxygenase isoforms in the striatum is miniscule whereas basal cGMP level in the striatum is the highest in the brain. Taken together, it is likely that the activity of sGC in its role in mediating GABA transmission and plasticity is primarily regulated by NO. In contrast to sGCβ1 KD, the mechanism underlying the reduction in GABA signaling in SPN collaterals after pharmacologic inhibition of NOS may not depend on transcriptional regulation, suggesting that multiple mechanisms mediate the effect of NO on GABA transmission. Several mechanisms have been proposed in the action of NO on GABA transmission and plasticity, including some that are independent of sGC (1, 2). To identify sGC-dependent effects of NO, we down-regulated sGC in striatal neurons. In line with our previous reports showing that sGC and cGMP mediate NO signaling in SPNs (6, 12), the down-regulation of VGAT levels and the impairment in GABA signaling in sGCβ1 KO clearly indicate that sGC plays a major role in the function of axon collaterals of SPNs. The reduction in cGMP levels in response to application of NO donor after loss of sGC in cultured neurons further supports the idea that sGC is a major target of NO. However, a role for NO that is independent of sGC in mediating GABA transmission cannot be...
Materials and Methods

A full description of the materials and methods can be found in SI Materials and Methods.

Animals.

Experiments were performed in accordance with National Institutes of Health guidelines for the care and use of laboratory animals, and all procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (11), and were approved by the Animal Care and Use Committees of the Rockefeller University, the Weill Medical College of Cornell University, and the Feinberg School of Medicine, Northwestern University. For viral injections, 7-wk-old mice (or 5-wk-old mice for electrophysiology studies) were anesthetized with ketamine/xylazine and were bilaterally injected with 1.5 μL of AAV into the dorsal striatum using a mouse atlas-integrated stereotaxic instrument (Leica). Coordinates were +2.05 mm, +0.25 mm, −3.96 mm lateral, anterior, and ventral from bregma according to the Paxinos and Franklin mouse brain atlas (2001) (32). Unless specified otherwise, assessment of the effect of the AAV was performed at 25 ± 3.2 d after injections in lysates of striata that were dissected between anterior +0.38 and 0.00, relative to bregma. All other in vivo studies are described in SI Materials and Methods.

RNA Isolation, Purification, and Analysis.

Striata and brainstem tissues from six Drd1-bacTRAP mice (CP-73), both males and females, were pooled to isolate RNA. RNA was isolated as previously shown (10, 11).

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