AMP A Receptor-Dependent H2O2 Generation in Striatal Medium Spiny Neurons But Not Dopamine Axons: One Source of a Retrograde Signal That Can Inhibit Dopamine Release

Marat V. Avshalumov,* Jyoti C. Patel,* and Margaret E. Rice

Departments of Neurosurgery and of Physiology and Neuroscience, New York University School of Medicine, New York, New York

Submitted 9 May 2008; accepted in final form 14 July 2008

Avshalumov MV, Patel JC, Rice ME. AMPA receptor-dependent H2O2 generation in striatal medium spiny neurons but not dopamine axons: one source of a retrograde signal that can inhibit dopamine release. J Neurophysiol 100: 1590–1601, 2008. First published July 16, 2008; doi:10.1152/jn.90548.2008. Dopamine-glutamatergic interactions in the striatum are critical for normal basal ganglia-mediated control of movement. Although regulation of glutamatergic transmission by dopamine is increasingly well understood, regulation of dopaminergic transmission by glutamate remains uncertain given the apparent absence of ionotropic glutamate receptors on dopaminergic axons in dorsal striatum. Indirect evidence suggests glutamatergic regulation of striatal dopamine release is mediated by a diffusible messenger, hydrogen peroxide (H2O2), generated downstream from glutamatergic AMPA receptors (AMPARs). The mechanism of H2O2-dependent inhibition of dopamine release involves activation of ATP-sensitive K+ (KATP) channels. However, the source of modulatory H2O2 is unknown. Here, we used whole cell recording, fluorescence imaging of H2O2, and voltammetric detection of evoked dopamine release in guinea pig striatal slices to examine contributions from medium spiny neurons (MSNs), the principal neurons of striatum, and dopamine axons to AMPAR-dependent H2O2 generation. Imaging studies of H2O2 generation in MSNs provide the first demonstration of AMPAR-dependent H2O2 generation in neurons in the complex brain-cell microenvironment of brain slices. Stimulation-induced increases in H2O2 in MSNs were prevented by GYKI-52466, an AMPAR antagonist, or catalase, an H2O2 metabolizing enzyme, but amplified by mercaptosuccinate (MCS), a glutathione peroxidase inhibitor. By contrast, dopamine release evoked by selective stimulation of dopamine axons was unaffected by GYKI-52466 or MCS, arguing against dopamine axons as a significant source of modulatory H2O2. Together, these findings suggest that glutamatergic regulation of dopamine release via AMPARs is mediated through retrograde signaling by diffusible H2O2 generated in striatal cells, including medium spiny neurons, rather than in dopamine axons.

INTRODUCTION

As a central component of the basal ganglia (Albin et al. 1989; Kemp and Powell 1971a), the striatum receives glutamatergic input from most of the cerebral cortex as well as from the thalamus (Berendse and Groenewegen 1990; Gerfen and Wilson 1996; Graybiel et al. 1994; Kemp and Powell 1971b; McGeorge and Faull 1989). This excitatory input forms asymmetric synapses on the heads of dendritic spines of medium spiny neurons (MSNs), which are the principal neurons of the striatum that integrate incoming cortical activity and encode striatal output (Graybiel et al. 1994; Wilson 1993). Importantly, MSNs also receive synaptic dopamine input from midbrain dopamine neurons (Freund et al. 1984; Smith and Bolam 1990). Converging glutamatergic and dopaminergic afferents control striatal network output at the level of individual spines to regulate motor and cognitive function (e.g., Cagniard et al. 2006; Costa et al. 2006). Despite the lack of direct synaptic associations between glutamatergic and dopaminergic axons (Freund et al. 1984; Smith and Bolam 1990), their apposition on MSN spines and their complementary functions suggest a reciprocal modulatory relationship.

Evidence for dopamine-dependent regulation of glutamate transmission is well established, including inhibition of glutamate release via D2 dopamine receptors on corticostriatal afferents (Bamford et al. 2004a,b; Cepeda et al. 2001). This regulation by dopamine occurs through volume transmission (Cragg and Rice 2004; Fuxe and Agnati 1991; Garris et al. 1994; Rice 2000; Rice and Cragg 2008) with synaptic spillover of dopamine facilitated by the location of dopamine transporters only on dopaminergic axons and its actions mediated by predominantly extrasynaptic dopamine receptors (Cragg and Rice 2004; Nirenberg et al. 1996; Rice and Cragg 2008; Sesack et al. 1994; Yung et al. 1995). How synaptically released glutamate regulates striatal dopamine release is much less clear. Glutamatergic transmission is primarily “hard-wired” with synapses surrounded by neuronal and glial transporters that limit glutamate spillover and ensure primary activation of subsynaptic ionotropic receptors (Barbour 2001; Bergles et al. 1999; Danbolt 2001; Galvan et al. 2006; Rusakov et al. 1999). When glutamate spillover does occur, e.g., after uptake inhibition, dopamine release can be inhibited via metabotropic glutamate receptors on dopaminergic axons (Zhang and Sulzer 2003). However, any effect of ionotropic glutamate-receptor activation on dopamine release must be indirect given that dopaminergic axons lack these receptors (Bernard and Bolam 1998; Bernard et al. 1997; Chen et al. 1998).

Indirect evidence suggests that glutamate-dependent regulation of striatal dopamine release involves a diffusible messenger, hydrogen peroxide (H2O2), which is generated downstream from glutamatergic AMPA receptors (AMPARs) and which activates ATP-sensitive K+ channels (KATP) to inhibit dopamine release (Avshalumov et al. 2003; Avshalumov and Rice 2003). The source of modulatory H2O2 is unknown, although the AMPAR dependence suggests that generation is unlikely to occur in dopaminergic axons. Here we tested the...
hypothesis that MSNs are an important source of modulatory H$_2$O$_2$ in striatum given that these cells constitute 95% of striatal neurons (Kemp and Powell 1971a) and express functional AMPARs (Bernard and Bolam 1998; Bernard et al. 1997; Carter and Sabatini 2004; Chen et al. 1998; Kita 1996). Using real-time fluorescence imaging of intracellular H$_2$O$_2$ in MSNs with simultaneous whole cell recording in striatal slices, we show that glutamate input to dorsolateral striatum acts via AMPARs to generate H$_2$O$_2$ in MSNs, which could provide a retrograde signal to inhibit axonal dopamine release. Companion voltammetric and imaging data indicate that dopaminergic axons do not contribute to the generation of modulatory H$_2$O$_2$.

**Methods**

All animal handling procedures were in accordance with the National Institutes of Health guidelines and were approved by the New York University School of Medicine Animal Care and Use Committee.

**Slice preparation**

Procedures for slice preparation were similar to previously published methods (Avshalumov et al. 2005; Bao et al. 2005; Koós and Tepper 1999). Young adult male guinea pigs (Hartley, 150–250 g) were deeply anesthetized with sodium pentobarbital (50 mg/kg ip) and perfused transcardially with ~30 mL of ice-cold modified artificial cerebrospinal fluid (ACSF) containing (in mM) 225 sucrose, 2.5 KCl, 0.5 CaCl$_2$, 7 MgSO$_4$, 28 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, 7 glucose, 1 ascorbate, and 3 pyruvate. After perfusion and decapitation, the brain was rapidly removed and immersed in this ice-cold modified ACSF for 1–2 min, then bisected and blocked before slicing on a Vibratome (Ted Pella, St. Louis, MO). Most whole cell recording and imaging studies were done in coronal slices. However, some slices were also cut in an angled parasagittal plane, at a 20–25° rostralateral angle to the midline to preserve nigrostriatal dopaminergic fibers entering the striatum from the median forebrain bundle (see Fig. 4A). It should be noted that this angle is roughly perpendicular to incoming thalamostriatal fibers (Smeal et al. 2007), so that this slice orientation permits selective stimulation of the nigrostriatal dopaminergic pathway. Angled parasagittal slices were used for all dopamine recording experiments.

**Visualized whole cell recording and H$_2$O$_2$ imaging**

Striatal slices (300 μm thickness) were kept for 30 min at 34°C in a holding solution containing (in mM) 125 NaCl, 2.5 KCl, 1.0 MgCl$_2$, 25 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, 25 glucose, 1 ascorbate, 3 pyruvate, and 2 CaCl$_2$ at pH 7.3–7.4, saturated with 95% O$_2$-5% CO$_2$. The holding solution was then allowed to cool slowly to room temperature over ~30 min before experimentation. For recording, slices were transferred to a submersion chamber (Warner Instruments LLC, Holliston, MA) maintained at 32°C and superfused at 1.2 mL/min with bicarbonate-buffered ACSF containing (in mM) 124 NaCl, 3.7 KCl, 26 NaHCO$_3$, 1.3 MgSO$_4$, 1.3 KH$_2$PO$_4$, 10 glucose, and 2.4 CaCl$_2$ and saturated with 95% O$_2$-5% CO$_2$.

MSNs in dorsolateral striatum were identified visually under infrared differential interference contrast (IR-DIC) microscopy using an Olympus BX51WI fixed-stage microscope (New York/New Jersey Scientific; Middlebush, NJ) with a ×40 water-immersion objective. Patch pipettes for whole cell recording and dye loading were pulled from borosilicate glass capillaries (1.5 mm OD, 0.86 mm ID) on a Flaming/Brown model P-97 micropipette puller (Sutter Instruments, Novato, CA). Pipettes had open tip diameters of <2 μm and resistances of 3–8 MΩ. The intracellular filling solution contained (in mM) 120 K-glucolate, 20 KCl, 2 MgCl$_2$, 10 Na-HEPES, 10 EGTA, 2 Na$_2$-ATP, and 0.2 GTP; pH adjusted to 7.2–7.3 with KOH, 280–290 mosM (Avshalumov et al. 2005; Bao et al. 2005; Koós and Tepper 1999). The intracellular solution also contained Alexa Red (0.1%) for cell visualization plus the fluorescent dye 2′7′-dichlorodihydrofluorescein (H$_2$DCF) diacetate (7 μM) for H$_2$O$_2$ imaging (Avshalumov et al. 2005, 2007).

Imaging of intracellular H$_2$O$_2$ was as described previously (Avshalumov et al. 2005; Bao et al. 2005). Briefly, H$_2$DCF diacetate was loaded into a given cell via the patch pipette used for simultaneous whole cell recording. Once a whole cell configuration was obtained, cell physiology was monitored for 15–20 min in current-clamp mode before images were taken to allow sufficient time for the dye to infiltrate the cell and cleavage by intracellular esterases to form H$_2$DCF; H$_2$DCF becomes fluorescent DCF after oxidation by H$_2$O$_2$ or other ROS (Avshalumov et al. 2005, 2007; Reynolds and Hastings 1995; Sah and Schwartz-Bloom 1999). Excitation wavelength for DCF was 488 nm with emission at 535 nm. To minimize DCF photobleaching, images were obtained at 500-ms intervals with 30-ns exposure using eight-frame averaging. Background fluorescence from an area adjacent to the recorded cell was subtracted from each averaged image for that cell. Fluorescence data are presented as [(stimulated intensity – basal)/(basal)] × 100%. Basal DCF fluorescence was taken as the average intensity recorded for 2 s immediately before stimulation. Stimulated intensity was determined from a 2-s average taken after the stimulus ended for comparison with the average basal fluorescence for each cell.

Activity-dependent H$_2$O$_2$ generation in MSNs was elicited using surface bipolar stimulating electrodes. In most experiments, local stimulation (<200 μm from a recorded cell) was used in coronal slices. However, angled parasagittal slices were used to compare the effects of local stimulation with stimulation of the nigrostriatal dopaminergic pathway. For these experiments, one bipolar electrode was positioned locally, then a second electrode was positioned >1.5 mm from a recorded cell with an orientation that was shown in voltammetric studies to elicit reliable dopamine release. Stimulation parameters were 30 pulses at 10 Hz with pulse duration of 100 μs and amplitude of 0.6–0.8 mA for local and 2–4 mA for pathway stimulation as optimized in studies of evoked dopamine release as described in the following text.

**Voltammetric monitoring of dopamine release**

All voltammetric recording of evoked dopamine release in dorsolateral striatum was done in angled parasagittal slices. Slices (400 μm) were maintained at room temperature for ≥1 h before experimentation in HEPES-buffered ACSF containing (in mM) 120 NaCl, 5 KCl, 20 NaHCO$_3$, 6.7 HEPES acid, 3.3 HEPES salt, 2 MgSO$_4$, 10 glucose, and 2 CaCl$_2$, saturated with 95% O$_2$-5% CO$_2$, which minimizes slice edema (MacGregor et al. 2001; Rice et al. 1997). Dopamine release was examined in submersed slices maintained at 32°C and superfused with the same bicarbonate-buffered ACSF used for whole cell recording and H$_2$O$_2$ imaging.

Evoked extracellular dopamine concentration ([DA$_e$]) was monitored with carbon-fiber microelectrodes (8 μm tip diameter, 30–50 μm length; made in-house using methods modified from Millar and Pelling 2001) and fast-scan cyclic voltammetry using a Millar voltammetric analyzer (available from Dr. Julian Millar at Queen Mary University of London, UK). Data acquisition and analysis were as described previously (Chen et al. 2001). Carbon-fiber microelectrodes were inserted in the dorsolateral striatum with the tip 50–100 μm below the slice surface. Surface bipolar stimulating electrodes were positioned to elicit optimal [DA$_e$] at a single recording site for both local and dopamine axon pathway stimulation (see Fig. 4). After 30-min equilibration in the slice chamber, dopamine release was evoked at 10-min intervals by alternating local and dopamine pathway stimulation (30 pulses, 10 Hz; 100-μs pulse duration). The stimulation intensity that evoked perimaximal [DA$_e$] was determined for each stimulation site; stimulus amplitude was 0.6–0.8 mA for local stimulation and 2–4 mA for dopamine pathway stimulation. Evoked [DA$_e$] was quantified by postexperimental calibration with known concentrations of dopamine at 32°C in all media used in a given experiment.

**J Neurophysiol • Vol. 100 • September 2008 • www.jn.org**
HPLC analysis of striatal dopamine tissue content

In separate experiments, the striatal dopamine tissue content of coronal slices (400 μm) was determined using HPLC with electrochemical detection as described previously (Chen et al. 2001). Slice pairs were allowed to equilibrate for 30 min at 32°C in bicarbonate-buffered ACSF before experimentation as in dopamine release studies. One slice of each pair was then incubated for a further 30 min at 32°C in bicarbonate-buffered ACSF alone while the other was incubated in ACSF containing a glutathione (GSH) peroxidase inhibitor mercaptosuccinate (MCS, 1 mM) (Avshalumov et al. 2003) for 30 min. After incubation, a sample of striatal tissue from each slice was weighed, frozen on dry ice, and stored at −80°C for subsequent HPLC analysis. On the day of analysis, samples were diluted 100-fold with ice-cold, deoxygenated mobile-phase, sonicated, spun at 13,000 rpm for 2 min, then the supernatant (10 μL) injected directly into the HPLC system for determination of dopamine content.

Drugs and chemicals

All experimental solutions were prepared immediately before use. MCS (as mercaptosuccinic acid) and components of all ACSF solutions used were from Sigma Chemical (St. Louis, MO). Catalase was from Calbiochem (San Diego, CA). For some experiments, catalase was heat-inactivated as described previously (Avshalumov et al. 2003). Other drugs, including AP5, 1-(4-aminophenyl)-4-methyl-7,8-methyleneedioxy-5H-2,3-benzodiazepine hydrochloride (GYKI-52466), and tetrodotoxin (TTX) were from Tocris Cookson (Ellisville, MO). Alexa Red and 2′,7′-H2DCF diacetate were from Invitrogen (Carlsbad, CA). Stock solutions of H2DCF diacetate were prepared in dimethylsulfoxide (DMSO) (Avshalumov et al. 2003, 2005); final DMSO levels were <0.1%, which alone had no effect on control responses.

Statistics

Data are expressed as means ± SE where n equals the number of cells for whole cell recording and imaging data or slices for dopamine release and dopamine content data. Significance of differences was assessed using paired or unpaired Student’s t-test for comparison of two groups as appropriate, one-way ANOVA for comparison of three groups, or two-way ANOVA for comparison of time versus drug-dependent effects.

RESULTS

MSN identification

Striatal MSNs were identified by their somatodendritic morphology and their electrophysiological characteristics in coronal or angled parasagittal brain slices; these characteristics were independent of plane of section. Recorded cells (n = 61) were not spontaneously active in vitro but did exhibit spike activity with depolarizing current injection as described previously (Bao et al. 2005); mean resting membrane potential was −77.0 ± 0.9 mV and input resistance was 78.1 ± 2.0 MΩ. Additionally, all neurons exhibited slow depolarization ramps in response to depolarizing currents and inward rectification with hyperpolarizing currents consistent with previously described properties of striatal MSNs (Bao et al. 2005; Kitai et al. 1979; Koós and Tepper 1999; Nisenbaum and Wilson 1995).

Stimulus-induced H2O2 generation in striatal MSNs

To monitor activity-dependent ROS generation, MSNs were loaded with H2DCF diacetate via the recording pipette; H2DCF becomes fluorescent DCF after oxidation by H2O2 or other ROS (Avshalumov et al. 2005, 2007; Reynolds and Hastings 1995; Sah and Schwartz-Bloom 1999). Although MSNs did not exhibit spontaneous activity, basal DCF fluorescence, reflecting tonic ROS production, was seen in all recorded MSNs (Fig. 1A). During local electrical stimulation, each pulse of the stimulus train (30 pulses, 10 Hz) elicited a single action potential in MSNs (Fig. 1A). This stimulus paradigm also produced a significant 25–35% increase in simultaneously recorded DCF fluorescence intensity (FI) in a majority of MSNs monitored under these conditions (P < 0.01; paired t-test vs. basal DCF FI; n = 7 of 11 recorded cells). In contrast, 4 of 11 cells showed no change in DCF FI (P > 0.05 paired t-test vs. basal) although physiological responses to stimulation were indistinguishable between these two groups as discussed further at the end of RESULTS. Interestingly, there was no intermediate response between these two extremes. When seen, increases in DCF FI were rapid, beginning after 5–10 stimulus pulses and reaching an average maximal level of 29 ± 1% (n = 7; Fig. 1, A and D). It should be noted that H2DCF is irreversibly activated by ROS, such that a plateau in DCF FI persists after stimulation, precluding evaluation of ROS clearance (e.g., Avshalumov et al. 2005).

Given that H2DCF can react with other ROS as well as H2O2, it was critical to ascertain whether or not the monitored increase in DCF FI was H2O2 dependent. Endogenous H2O2 levels are regulated primarily by the cellular enzymes GSH peroxidase and catalase (Cohen 1994; Stults et al. 1977). Therefore we examined whether stimulus-induced H2O2 generation in striatal MSNs could be modulated by manipulation of these peroxidase enzymes. When GSH peroxidase activity was inhibited by MCS (1 mM) (Avshalumov et al. 2003, 2005), the stimulus-induced increase in DCF FI was amplified to nearly twice that seen under control conditions (Fig. 1, B and D; P < 0.001 vs. control, n = 7 per group). In striking contrast to the control population, all MSNs recorded in the presence of MCS showed an increase in DCF FI during local stimulation, suggesting that the antioxidant status of individual MSNs dictates the magnitude of activity-dependent H2O2 levels. Confirming that H2O2 is the primary ROS generated in MSNs during local stimulation, no increase in DCF FI was seen in any recorded MSN in the presence of catalase (n = 5; 500 U/mL) (Avshalumov et al. 2003), although action potential generation was unaltered (Fig. 1, C and D). By contrast, a typical proportion of MSNs (3 of 5) showed an increase in DCF FI with stimulation in the presence of heat-inactivated catalase (26 ± 1%; P < 0.01 vs. basal; n = 3). These data not only confirmed that H2O2 was the monitored ROS in MSNs but also showed that activity-dependent H2O2 levels are governed by cellular peroxidase activity.

Stimulus-induced H2O2 generation in striatal MSNs requires AMPAR activation

In the presence of the Na+ channel blocker TTX (1 μM) (Avshalumov et al. 2005), local stimulation in dorsolateral striatum failed to elicit either action potentials or an associated increase in intracellular H2O2 in any recorded MSN (n = 5; Fig. 2, A and C), demonstrating that H2O2 generation requires action potentials. To determine whether the effect of TTX was primarily the result of inhibition of spike generation per se or inhibition of synaptic transmitter release, we compared the
effect of depolarizing current injection in individual MSNs on DCF FI with that evoked by local stimulation. Using a current injection paradigm designed to mimic the effect of local stimulation (30 pulses, 10 Hz), we found no detectable increase in endogenous intracellular H$_2$O$_2$ levels despite the induction of spike firing of each recorded MSN ($n = 5$; Fig. 2B). In the same cells, however, subsequent local stimulation caused the usual $\sim50\%$ increase in DCF FI (Fig. 2B and C). Taken together, these data suggest that activity-dependent generation of modulatory H$_2$O$_2$ in striatum requires synaptic input.

Previous studies in cultured neurons indicate that intracellular ROS production increases in response to exogenous glutamate agonists (Bindokas et al. 1996; Carriedo et al. 2001; Dugan et al. 1995; Lafon-Cazal et al. 1993; Reynolds and Hastings 1995). We therefore examined whether endogenous glutamate released during local pulse-train stimulation was required for H$_2$O$_2$ generation in striatal MSNs. Striatal MSNs express both NMDA receptors (NMDARs) and AMPARs (Bernard and Bolam 1998; Chen et al. 1998). However, excitation is largely mediated by AMPARs rather than NMDARs with mild

![FIG. 1. Activity-dependent generation of endogenous H$_2$O$_2$ in striatal medium spiny neurons (MSNs). A–C: representative examples of intracellular H$_2$O$_2$ (top) and membrane voltage ($V_{memb}$; bottom) monitored simultaneously in identified MSNs during local pulse-train stimulation (30 pulses, 10 Hz). The time course of stimulus-induced changes in dichlorofluorescein (DCF) fluorescence intensity (FI) is shown along with pseudo-color images of DCF FI recorded under basal conditions and at the end of stimulation (scale bar = 20 \mu m). A: stimulus-induced increase in DCF FI in an MSN during local stimulation; under control conditions, 7 of 11 MSNs showed a significant increase in DCF FI ($P < 0.001$ vs. basal). Simultaneous current-clamp recording indicated that a single action potential was generated with each stimulus pulse in all recorded MSNs. B: inhibition of glutathione (GSH) peroxidase by mercaptoproline (MCS, 1 mM) amplified the stimulus-evoked increases in DCF FI, consistent with selective detection of H$_2$O$_2$, with no effect on action potential generation in recorded MSNs. In the presence of MCS, 7 of 7 MSNs showed a significant increase in DCF FI ($P < 0.001$). C: the presence of the H$_2$O$_2$ metabolizing enzyme catalase (Cat; 500 U/mL) did not alter spike generation during stimulation but prevented the stimulus-induced increase in DCF FI in 5 of 5 MSNs. D: average stimulus-induced changes in DCF FI in H$_2$O$_2$ source MSNs under control conditions (Con; $n = 7$), in the presence of MCS ($n = 7$), or in the presence of catalase ($n = 5$; ***$P < 0.01$ vs. basal; ****$P < 0.001$ vs. basal). The increase in DCF FI in MCS was nearly twofold greater than under control conditions, whereas the presence of catalase markedly attenuated the usual control response (###$P < 0.001$ vs. control), confirming the H$_2$O$_2$ dependence of the evoked increases in DCF FI.]

![FIG. 2. Endogenous H$_2$O$_2$ generation in striatal MSNs requires coherent synaptic activation. A: intracellular H$_2$O$_2$ (top) and membrane voltage ($V_{memb}$; bottom) monitored simultaneously in MSNs during local pulse-train stimulation (30 pulses, 10 Hz) in tetrodotoxin (TTX, 1 \mu M). In the presence of TTX, stimulus-induced action potential generation was prevented and no increase in DCF FI was seen in any recorded MSN (5 of 5). B: current injection (30 pulses, 10 Hz; pulse amplitude: 5–10 nA; pulse duration: 100 \mu s) alone elicited action potentials in individual MSNs, but no increase in DCF FI was detected in any MSN examined ($n = 5$), whereas subsequent local stimulation (30 pulses, 10 Hz) caused the usual 30% increase in DCF FI in the same cells ($n = 5; P < 0.001$ vs. basal). This suggests that synaptic activation of MSNs, rather than action potential generation alone, is required to generate modulatory H$_2$O$_2$. C: average DCF FI in the presence of TTX ($n = 5$) or with current injection with subsequent local stimulation, as described for panel B ($n = 5$; ***$P < 0.001$ vs. basal).]
stimulation (Jiang and North 1991; Kita 1996; Pennartz et al. 1991). Consistent with these data, blockade of AMPARs with GYKI-52466 (50–100 µM) (Avshalumov et al. 2003) prevented both the generation of action potentials and stimulus-induced increases in DCF FI in all recorded MSNs during local stimulation (n = 7; Fig. 3, A and C). By contrast, stimulus-induced action potentials were unaltered by blockade of NMDARs by AP5 (100 µM) (Avshalumov et al. 2003) and an increase in DCF FI persisted in recorded MSNs (n = 5, Fig. 3, B and C). These data are the first to show that endogenous glutamate acting via AMPARs leads to H₂O₂ generation in CNS neurons.

**Does activation of dopaminergic axons produce autoinhibitory H₂O₂?**

Dopaminergic axons are another potential source of modulatory H₂O₂, either directly as a consequence of increased mitochondrial oxygen consumption during action potential generation (Boveris and Chance 1973; Kennedy et al. 1992) and/or indirectly via dopamine metabolism or autoxidation (Berman and Hastings 1999; Cohen 1994; Kulagina and Michael 2003) or via AMPAR activation of MSNs by glutamate co-released from dopaminergic axon terminals (Chuhma et al. 2004; Kaneko et al. 1990; Sulzer et al. 1998; Trudeau 2004).

We examined the role of these possible contributing factors to H₂O₂-dependent suppression of striatal dopamine release using angled parasagittal slices in which dopaminergic axon tracts within the striatum are sufficiently preserved to permit dopamine pathway stimulation 1.5–2 mm distal to a recording site. Dopamine release evoked by alternating local and pathway pulse-train stimulation (30 pulses, 10 Hz) was monitored voltammetrically at a single recording site with a carbon-fiber microelectrode (Fig. 4A) and identified by the single oxidation and single reduction peak potentials that define the signature voltammogram for dopamine (Fig. 4B, insets). Dopamine release evoked by distal stimulation was dopamine pathway specific; an increase in [DA]ₐ was only seen when the pathway-stimulating electrode was positioned ventrocaudally to the recording electrode but not from sites that were perpendicular to this location. The average [DA]ₐ evoked by local stimulation was ~40% higher than that evoked by dopamine pathway stimulation (Fig. 4, C and D; P < 0.001; n = 12), reflecting the efficacy of local stimulation to activate most, if not all, dopaminergic axons surrounding a recording site, contrasted with pathway stimulation, which activates dopaminergic axons that diverge from the stimulus site.

To elucidate whether selective activation of dopaminergic axons generates H₂O₂ that might serve as an autoinhibitory signal to regulate dopamine release, we amplified endogenous H₂O₂ levels by inhibiting GSH peroxidase using MCS, then compared the effect on [DA]ₐ evoked locally and by pathway stimulation. Consistent with the elevation of H₂O₂ levels in MSNs during local stimulation in the presence of MCS (Fig. 1), locally evoked [DA]ₐ was suppressed throughout the stimulus after 30 min exposure to MCS (2-way ANOVA, P < 0.001 vs. same-site control, n = 6; Fig. 5, A and B) as described previously (Avshalumov et al. 2003). Release suppression was reversible on MCS washout (not shown). In contrast to the effect of MCS on locally evoked [DA]ₐ, however, MCS did not alter either the peak amplitude or the time course of [DA]ₐ evoked at the same recording site by dopamine pathway stimulation (2-way ANOVA, P > 0.05, n = 6; Fig. 5, A and B). This implies the absence of modulatory H₂O₂ generation during selective stimulation of dopaminergic axons, including that from dopamine metabolism or autoxidation.

The lack of effect of GSH peroxidase inhibition on dopamine pathway-evoked [DA]ₐ also argues against the possibility that acutely elevated H₂O₂ levels might deplete the releasable pool of dopamine, e.g., by direct oxidation. Separate companion studies of striatal dopamine content confirmed that dopamine levels were unaltered by exposure to MCS (1 mM, 30 min) with dopamine content of 55 ± 3 nmol/g tissue wet weight (n = 16) in slices exposed to MCS versus 58 ± 1 nmol/g (n = 16) in control slices (P > 0.05 MCS vs. control, unpaired t-test).

Given evidence for co-release of glutamate and dopamine from dopaminergic neurons in culture and from mesolimbic dopaminergic input to ventral striatum (nucleus accumbens) in brain slices (Chuhma et al. 2004; Kaneko et al. 1990; Sulzer et al. 1998; Trudeau 2004), we next examined whether co-

---

**FIG. 3.** Glutamate-dependent H₂O₂ generation in striatal MSNs requires AMPAR activation. A and B: intracellular H₂O₂ (top) and membrane voltage (V_memb; bottom) monitored simultaneously in MSNs during local pulse-train stimulation (30 pulses, 10 Hz). Time course of stimulus-induced changes in DCF FI are shown with pseudocolor images of DCF FI recorded under basal conditions and at the end of stimulation (scale bar = 20 µm). A: the usual stimulus-induced increase in DCF FI in MSNs was prevented by GYKI-52466 (50–100 µM), an AMPAR antagonist as were stimulus-evoked action potentials monitored during simultaneous current-clamp recording (n = 7; P > 0.05 vs. basal). B: blockade of NMDARs by AP5 (100 µM) had no effect on either DCF FI or action potential generation in recorded MSNs with 5 of 5 MSNs showing a significant increase in DCF FI (P < 0.001 vs. basal). C: summary of average changes in DCF-FI in MSNs in the presence of GYKI (n = 7) or AP5 (n = 5) (**P < 0.001 vs. basal).
released glutamate during stimulation of nigrostriatal dopaminergic input to dorsolateral striatum might contribute to glutamate-dependent H$_2$O$_2$ generation and consequent dopamine release modulation. Previous studies show that striatal dopamine release evoked by local pulse-train stimulation is suppressed by concurrent glutamate release acting at AMPARs (Avshalumov et al. 2003). We therefore compared the effect of AMPAR blockade by GYKI-52466 on [DA]$_o$ evoked by local versus dopamine pathway stimulation (30 pulses, 10 Hz). As previously, GYKI-52466 caused an increase of $\sim$70% in peak amplitude of [DA]$_o$ evoked by local stimulation (Fig. 5, C and D) with amplification of evoked [DA]$_o$ throughout the pulse train (2-way ANOVA, $P < 0.001$ vs. same-site control, $n = 6$), confirming AMPAR-dependent modulation of dopamine release. At the same recording site, however, GYKI-52466 had no effect on the amplitude of [DA]$_o$ evoked by dopamine pathway stimulation (Fig. 5, C and D; 2-way ANOVA, $P > 0.05$ vs. control, $n = 6$). These data suggest that if co-release of dopamine and glutamate from dopaminergic axons does occur in dorsolateral striatum, this source of glutamate input does not contribute to AMPAR-mediated suppression of dopamine release. These data also support the absence of concurrent activation of thalamostriatal input during dopamine pathway stimulation.

**Lack of H$_2$O$_2$ generation in MSNs during dopamine pathway stimulation**

To examine possible contributions from concurrent thalamostriatal activation in angled parasagittal slices directly and to test whether H$_2$O$_2$ generation consequent to stimulation of dopaminergic axons might contribute to elevated H$_2$O$_2$ levels in striatal MSNs, we compared DCF FI in MSNs during local versus dopamine pathway stimulation. Using the same orientation of stimulating and recording electrodes found to elicit reliable dopamine release (Fig. 4), dopamine pathway stimulation (30 pulses, 10 Hz) failed to evoke action potentials in any recorded MSN ($n = 13$; Fig. 6). This not only confirms the
absence of thalamostriatal pathway activation but also argues further against glutamate co-release from dopamine axons. Moreover simultaneously recorded DCF FI did not differ from basal DCF FI in these cells (n/H11005 13, P/H11022 0.05 vs. basal). The lack of detectable H2O2 elevation in MSNs, here used as “reporter cells,” suggests minimal production of diffusible H2O2 during selective dopamine axon stimulation. By contrast, subsequent local pulse-train stimulation elicited a single action potential for each stimulus pulse delivered in the same MSNs (Fig. 6), accompanied by an increase of 31 % in DCF FI in 10 of 13 recorded cells (P/H11021 0.001 vs. basal; n/H11005 10).

**H2O2 source versus nonsource MSNs**

Throughout these studies, a population of MSNs showed no detectable increase in DCF FI during local stimulation under control conditions or conditions in which action potential generation was unaltered (e.g., in heat-inactivated catalase). Of 39 cells recorded under these conditions, 30 produced a detectable increase in H2O2 (77%), whereas 9 did not (23%). This implies that a majority of MSNs are H2O2 “source” cells whereas a smaller population are “nonsource” (or possibly “sink”) cells. The occurrence of nonsource cells was not simply a technical artifact from insufficient dye loading because exogenously applied H2O2 10 Hz, 3 s

**FIG. 5.** Modulatory H2O2 is not generated in dopaminergic axons. A: average [DA]o vs. time profiles evoked at a single site by alternating local and pathway stimulation (30 pulses, 10 Hz) in the absence and presence of the GSH peroxidase inhibitor mercaptosuccinate (MCS; 1 mM; n = 6). B: summary of the effect of MCS on peak [DA]o at a given site evoked by local vs. dopamine pathway stimulation; control peak evoked [DA]o was taken as 100%. Increasing endogenous H2O2 availability by inhibiting GSH peroxidase caused a significant decrease in [DA]o evoked by local stimulation but had no effect on pathway evoked [DA]o (n = 6; ***P < 0.001 vs. local control). C: average [DA]o vs. time profiles evoked at a single site by alternating local and pathway stimulation (30 pulses, 10 Hz) in the absence and presence of an AMPAR blocker, GYKI-52466 (GYKI; 50–100 µM; n = 6). D: summary of the effect of GYKI on peak [DA]o at a given site evoked by local and pathway stimulation; control peak evoked [DA]o is taken as 100%. Blockade of AMPARs caused a significant increase in [DA]o evoked by local stimulation but had no effect on that evoked by selective stimulation of dopaminergic axons (n = 6; ***P < 0.001 vs. local control).

**FIG. 6.** Absence of detectable H2O2 generation in MSNs during dopaminergic axon pathway stimulation. Representative example of H2O2 imaging (top) and simultaneous current-clamp (bottom) recording in an MSN during pathway and local pulse-train (30 pulses, 10 Hz) stimulation; the break in the current-clamp record indicates an interval of 2 min. Corresponding pseudocolor DCF FI images were taken at the time points indicated by the arrows; scale bars = 20 µm. Pathway stimulation of dopaminergic axons did not induce MSN firing or a change in DCF FI, whereas subsequent local stimulation induced firing of action potentials and ~30% increase in DCF FI in these same cells (n = 7).
caused an increase in DCF FI that was typically >70% of basal in these cells and was similar to that seen in source MSNs (not illustrated). A comparison of basic electrophysiological properties in subpopulations of each group indicated no difference in resting membrane potential (−77.2 ± 1.1 mV source vs. −76.2 ± 1.5 mV nonsource) or input resistance (79.9 ± 2.3 MΩ source vs. 72.9 ± 3.6 MΩ nonsource; unpaired t-test, P > 0.05 for both comparisons, n = 15 source and 5 nonsource MSNs). Excitability also appeared similar in both source and nonsource cells, given that local stimulation evoked action potentials that faithfully followed stimulation frequency in both populations. Last the viability of both populations was similar with similar recording times possible for both groups. Importantly, however, when GSH peroxidase was inhibited by MCS (e.g., Fig. 1B), all MSNs tested (7/7) showed a significant increase in DCF FI, as noted in the preceding text. This implies that differences in peroxidase enzyme activity in MSN subpopulations determine intracellular \( \text{H}_2\text{O}_2 \) levels in a given cell and, therefore whether the cell will be a source of glutamatergic-dependent, modulatory \( \text{H}_2\text{O}_2 \).

**Discussion**

How synaptically released transmitters affect adjacent release sites that lack discrete point-to-point synaptic connections, like dopaminergic and glutamatergic inputs to striatal MSNs, is a long-standing question. In the case of dopamine, the answer is that it acts by volume transmission (Cragg and Rice 2004; Fuxe and Agnati 1991; Garris et al. 1994; Rice 2000; Rice and Cragg 2008). However, glutamate acts primarily within a synapse (Barbour 2001; Rusakov et al. 1999) with spillover limited by avid uptake. Thus whether synaptically released glutamate acting at ionotropic glutamate receptors regulates dopamine release is unresolved. We show here that glutamatergic excitation of AMPARs in striatal MSNs increases generation of the diffusible signaling molecule, \( \text{H}_2\text{O}_2 \), in a large population of these cells. This is the first direct demonstration of \( \text{H}_2\text{O}_2 \) generation in neurons by *endogenous* glutamate. By contrast, we found no evidence for generation of modulatory \( \text{H}_2\text{O}_2 \) by dopamine axons or released dopamine. Together, these data support a model of glutamatergic regulation of striatal dopamine release that is mediated in large part by AMPAR-dependent \( \text{H}_2\text{O}_2 \) generated as a retrograde messenger in striatal neurons, including MSNs, rather than dopamine axons.

**\( \text{H}_2\text{O}_2 \) as a signaling molecule**

In contrast to other ROS, \( \text{H}_2\text{O}_2 \) is neither a free radical nor an ion, which limits its reactivity (Cohen 1994) and increases its membrane permeability (Bienart et al. 2006, 2007; Makino et al. 2004; Ramasarma 1982), so that it is well-suited as a diffusible messenger. Recent evidence suggests that cell-specific membrane permeability to \( \text{H}_2\text{O}_2 \) governs its efflux and entry (Bienart et al. 2007; Makino et al. 2004). Cellular levels, therefore, reflect the balance among generation, primarily by mitochondrial respiration (Boveris and Chance 1973; Dugan et al. 1995; Kennedy et al. 1992; Liu et al. 2002), metabolism, primarily by GSH peroxidase and catalase (Cohen 1994; Stults et al. 1977) but also peroxiredoxins (Hofmann et al. 2002; Rhee et al. 2001), and \( \text{H}_2\text{O}_2 \) diffusion into and out of cells (Makino et al. 2004). The role of peroxide metabolism in managing cellular \( \text{H}_2\text{O}_2 \) levels in MSNs was shown by the marked amplification of activity-dependent \( \text{H}_2\text{O}_2 \) levels in all recorded MSNs after GSH peroxidase inhibition (Fig. 1, B and D). The finding that *all* recorded MSNs showed a stimulation-induced increase in \( \text{H}_2\text{O}_2 \) in the presence of MCS also suggests that GSH peroxidase helps determine whether individual MSNs are \( \text{H}_2\text{O}_2 \)-source or nonsource cells. Importantly, demonstration that AMPAR-dependent generation of modulatory \( \text{H}_2\text{O}_2 \) occurs in MSNs (Fig. 3A), but not dopaminergic axons (Fig. 5), further indicates that \( \text{H}_2\text{O}_2 \) must diffuse from an external cellular source to inhibit axonal dopamine release.

No generation of modulatory \( \text{H}_2\text{O}_2 \) by dopamine axons

In our initial report showing that endogenously produced \( \text{H}_2\text{O}_2 \) inhibited dopamine release, we suggested that dopamine axons might be the primary source of activity-dependent \( \text{H}_2\text{O}_2 \) (Chen et al. 2001), given the abundance of mitochondria within a few hundred nanometers of dopamine axon terminals (Nirenberg et al. 1997). In that case, \( \text{H}_2\text{O}_2 \) would be an autoinhibitor of dopamine release as it is in dopamine cell bodies (Avshalumov et al. 2005). However, the hypothesis of *direct* generation of modulatory \( \text{H}_2\text{O}_2 \) by dopamine axons is not supported by our subsequent work, including the present studies. Most obviously, we found that dynamic regulation of dopamine release by endogenous \( \text{H}_2\text{O}_2 \) requires AMPAR activation (Avshalumov et al. 2003). As noted in the introduction, this implies that dopamine axons cannot be the primary source of modulatory \( \text{H}_2\text{O}_2 \) because they lack AMPARs (Bernard and Bolam 1998; Bernard et al. 1997; Chen et al. 1998). Indeed GSH peroxidase inhibition by MCS has no effect on locally evoked \([\text{DA}]_o\) when AMPARs are blocked (Avshalumov et al. 2003), indicating that there is no remaining \( \text{H}_2\text{O}_2 \) signal to amplify. The present finding that MCS has no effect on pathway-evoked \([\text{DA}]_o\) (Fig. 5A) confirms that there is little, if any, direct AMPAR-independent contribution from dopamine axons to the generation of modulatory \( \text{H}_2\text{O}_2 \). These and other data also imply that there are no *indirect* contributions to dynamically generated \( \text{H}_2\text{O}_2 \) from metabolism or autooxidation of released dopamine (Berman and Hastings 1999; Cohen 1994; Kulagina and Michael 2003) or from glutamate co-released from dopamine axons (Chuhma et al. 2004; Kaneko et al. 1990; Sulzer et al. 1998; Trudeau 2004). The findings that MCS has no effect on pathway-evoked dopamine release (Fig. 5A) or on locally evoked release when AMPARs are blocked and evoked \([\text{DA}]_o\) is nearly twofold higher than control (Avshalumov et al. 2003) argue against a modulatory role for \( \text{H}_2\text{O}_2 \) formed from released dopamine. Indirect support for this argument comes from the absence of detectable elevation in DCF FI in MSNs during selective dopamine pathway stimulation, which could have reflected \( \text{H}_2\text{O}_2 \) diffusion from dopamine axons (Fig. 6). The inability of dopamine pathway stimulation to increase DCF FI or to alter MSN physiology (Fig. 6) coupled with the absence of an effect of AMPAR blockade on dopamine pathway-evoked \([\text{DA}]_o\) (Fig. 5C) further shows that glutamate co-released from dopamine axons does not contribute to AMPAR-dependent generation of modulatory \( \text{H}_2\text{O}_2 \).

Glutamate-dependent generation of \( \text{H}_2\text{O}_2 \) in MSNs

In contrast to the lack of evidence for self-regulation of dopamine release by \( \text{H}_2\text{O}_2 \) generated in dopamine axons, the
present studies clearly demonstrate glutamate-dependent H$_2$O$_2$ generation in MSNs. The findings and approaches used differ in three main ways from earlier work on glutamate-receptor agonist-induced increases in H$_2$O$_2$ or other ROS levels in cultured neurons (e.g., Bindokas et al. 1996; Carriedo et al. 2001; Dugan et al. 1995; Lafon-Cazal et al. 1993; Reynolds and Hastings 1995). First, in previous studies, exogenous glutamate agonists were used to elicit increases in ROS, whereas here endogenously released glutamate was the trigger. Second, the earlier focus was on glutamate neurotoxicity, whereas the present studies demonstrate that AMPAR-dependent H$_2$O$_2$ generation is a component of normal glutamate signaling in striatum. Third, because CNS neurons in culture lack glia to provide glutamate uptake and limit excitotoxicity, as well as the glial antioxidant network that limits oxidative damage (e.g., Avshalumov et al. 2004), it is surprising and significant that increases in intracellular H$_2$O$_2$ in MSNs also occur in the complex microenvironment of brain slices during AMPAR activation by endogenous glutamate.

It is also notable that action potential generation alone in MSNs was not sufficient to generate H$_2$O$_2$. Although the factors underlying this difference are not yet known, a requirement for glutamatergic input has been reported previously for another class of diffusible messengers, the endocannabinoids. Kreitzer and Malenka (2005) found that detectable endocannabinoid release from MSNs when postsynaptic depolarization was paired with presynaptic stimulation but not with postsynaptic depolarization alone.

Are MSNs the only source of dynamic, modulatory H$_2$O$_2$ generation? The role of other striatal cells has not yet been examined; however, it is likely that MSNs are an important source of modulatory H$_2$O$_2$ given that MSNs constitute 90–95% of the neuron population of the striatum (Kemp and Powell 1971a). Moreover, glutamate synapses are closely opposed to dopaminergic synapses on the dendrites of MSNs (Freund et al. 1984; Smith and Bolam 1990) and are therefore ideally positioned to modulate dopamine release via postsynaptically generated H$_2$O$_2$. Consistent with this anatomical evidence, stimulated H$_2$O$_2$ generation in MSNs is dependent on AMPAR activation, amplified by inhibition of GSH peroxidase, and eliminated by catalase (Figs. 1 and 3) in a pattern consistent with the previously reported consequences of these manipulations on dopamine release (Avshalumov et al. 2003). Thus H$_2$O$_2$ produced in MSNs alone could be sufficient to mediate glutamate-dependent regulation of dopamine release. Of course, contributions from the remaining 5–10% of striatal neurons could also occur as most of these express AMPAR subunits (Deng et al. 2007). Striatal glia might contribute, as well, with the caveat that although cultured striatal astrocytes express AMPAR protein (Fan et al. 1999), there are no reports of functional consequences of AMPAR activation in these cells in striatum.

How does H$_2$O$_2$ inhibit dopamine release? We have reported previously that H$_2$O$_2$ signaling in the striatum requires the activation of K$_{ATP}$ channels (Avshalumov and Rice 2003; Avshalumov et al. 2003), which are found throughout the nigrostriatal dopamine pathway (Avshalumov et al. 2003, 2005; Liss et al. 1999; Mourre et al. 1989; Xia and Haddad 1991; Zini et al. 1993). Although other H$_2$O$_2$ sensing systems from phosphatases and kinases to transcription factors can also regulate cell function (Finkel et al. 2003; Kamsler and Segal 2004; Kishida and Klann 2007; Rhee 2006; Rhee et al. 2005; Veal et al. 2007), these processes are very slow (minutes to hours) compared with the rapid, dynamic H$_2$O$_2$ regulation of striatal dopamine release which occurs on a subsecond time scale.

Based on these findings, we propose a model of glutamate-dependent modulation of axonal dopamine by AMPAR-dependent H$_2$O$_2$ generation in MSNs in which AMPAR activation of dendrites and somata of these cells leads to generation of H$_2$O$_2$ that diffuses to adjacent dopamine axons and inhibits dopamine release via opening of K$_{ATP}$ channels (Fig. 7, center). When AMPARs are blocked (+GYKI), H$_2$O$_2$-dependent regulation of dopamine release via K$_{ATP}$ channels is lost (Avshalumov and Rice 2003), and dopamine release is enhanced (Fig. 7, left). Conversely, when activity-dependent levels of H$_2$O$_2$ are amplified by inhibiting GSH peroxidase with MCS, this leads to enhanced H$_2$O$_2$-dependent K$_{ATP}$-channel activation (Avshalumov and Rice 2003), and further suppression of dopamine release (Fig. 7, right).

Conclusions and implications

What are the implications of diffusible H$_2$O$_2$ as the mediator of striatal glutamate-dopamine interactions? As an inhibitory intermediate, endogenously generated H$_2$O$_2$ reverses conventional glutamatergic excitation and leads to inhibition of dopamine release via H$_2$O$_2$-sensitive K$_{ATP}$ channels (Avshalumov et al. 2003; 2005; Smith and Bolam 1990) and expressed in striatal astrocytes (Fan et al. 1999), leading to further suppression of dopamine release.
and Rice 2003). Generation of detectable levels of H$_2$O$_2$ in a majority of MSNs following AMPAR activation would necessarily contribute to dopamine release inhibition given that this diffusible messenger will readily leave the cell in which it is produced. It would be expected that H$_2$O$_2$ is produced in all MSNs, and potentially all AMPAR-expressing cells, during glutamatergic activation. Whether a particular MSN or other cell is an H$_2$O$_2$ source, however, apparently depends largely on the peroxidase activity in that cell, inasmuch that all recorded MSNs showed an increase in DCF FI when endogenous peroxidase was inhibited, whereas stimulus-evoked H$_2$O$_2$ levels were kept below detection limits in ~25% of recorded MSNs under control conditions. Does detection indicate functionally relevant levels of H$_2$O$_2$? Our previous studies of the effect of endogenous H$_2$O$_2$ on the physiology of dopaminergic neurons in the substantia nigra indicate that the answer is yes. In those studies, we compared increases in DCF FI when endogenous H$_2$O$_2$ was amplified by increasing concentrations of the GSH peroxidase inhibitor, MCS. Low concentration of MCS, even those sufficient to cause a significant increase in DCF FI, had no effect on $K_{\text{ATP}}$ channels in dopamine neurons, implying that there is a threshold for $K_{\text{ATP}}$ channel modulation (i.e., a functional H$_2$O$_2$ concentration) that is above DCF detection limits (Avshalumov et al. 2005). Thus DCF sensitivity to H$_2$O$_2$ appears to be even greater than the sensitivity of $K_{\text{ATP}}$ channels to endogenous H$_2$O$_2$ levels. These data therefore imply that glutamatergic activation of source MSNs could produce levels of H$_2$O$_2$ sufficient to inhibit nigrostriatal dopamine input to those cells (Fig. 7), whereas nonsource MSNs lack this capacity. This would also hold true for other striatal cells that might serve as sources of H$_2$O$_2$.

Differential regulation of H$_2$O$_2$ generation in MSNs could be important physiologically, given differential dopaminergic regulation of striatonigral versus striatopallidal MSNs (Surmeier et al. 2007). For example, in striatonigral MSNs in which D1 dopamine-receptor activation enhances responsiveness to glutamatergic input (Albin et al. 1989; Surmeier et al. 2007), a consequent increase in H$_2$O$_2$ generation would provide an inhibitory signal to decrease dopamine input, which would then reverse D1-enhanced excitability in a reciprocal glutamate-dopamine feedback loop. By contrast, in striatopallidal MSNs, D2-receptor activation decreases excitability. A consequent decrease in H$_2$O$_2$ generation would lead to enhanced local dopamine release, resulting in further D2-receptor-mediated inhibition of MSNs excitability. The absence of activity-dependent H$_2$O$_2$ elevation in those MSNs, for example, would avoid an endless negative feedback process.

Last, glutamate-dopamine dysregulation has been implicated as a causal factor in Parkinson’s disease (Bevan et al. 2006; Chase and Oh 2000), Huntington’s disease (Di Filippo et al. 2007), schizophrenia (Sawa and Snyder 2002; Thompson et al. 2004), and addiction (Hyman and Malenka 2001; Kelley 2004). Oxidative stress is also linked causally to several of these disorders (Browne and Beal 2006; Orth and Schapira 2002; Klein and Schlossmacher 2006; Tosic et al. 2006). The finding that AMPAR activation in MSNs by endogenously release glutamate leads to enhanced H$_2$O$_2$ generation provides a new starting point for understanding glutamate-dopamine function and dysfunction.

ACKNOWLEDGMENTS

We are grateful to A. Quyyumi for HPLC analysis, M. L. Chao for immunocytochemistry, C. Nicholson for assistance with photography, and C. R. Lee and Z. Sidló for insightful feedback during the preparation of this report.

Present address of M. V. Avshalumov: Dept. of Neurosurgery, Mount Sinai Medical Center, New York, USA.

GRANTS

The work was funded by National Institute of Neurological Disorders and Stroke Grant NS-36362 and the Attilio and Olympia Ricciardi Research Fund.

REFERENCES


Avshalumov MV, Rice ME. Activation of ATP-sensitive K$^+$ ($K_{\text{ATP}}$) channels by H$_2$O$_2$ underlies glutamate-dependent inhibition of striatal dopamine release. Proc Natl Acad Sci USA 100: 11729–11734, 2003.


Bernard V, Bolam JP. Subcellular and subynaptic distribution of the NR1 subunit of the NMDA receptor in the neostriatum and globus pallidus of the rat: colocalization at synapses with the GluR2/3 subunit of the AMPA receptor. Eur J Neurosci 10: 3721–3738, 1998.


M. V. AVSHALUMOV, J. C. PATEL, AND M. E. RICE


