Dopamine Modulation of Membrane Excitability in Striatal Spiny Neurons is Altered in DARPP-32 Knockout Mice

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ABSTRACT

The phosphoprotein DARPP-32 (dopamine and cAMP-regulated phosphoprotein 32 kDa) plays a central role in mediating the actions of a variety of neurotransmitters in medium spiny neurons of the striatum (Greengard, 1990; Fienberg et al., 1998). This study examines D1 and D2 dopamine (DA) agonist effects on the membrane properties of identified striatal neurons recorded in slices obtained from wild-type and DARPP-32-knockout mice. In wild-type spiny cells, DA D1 receptor activation decreased cell excitability, causing a 58.8 ± 13.5% increase in rheobase current required to evoke spike discharge. In contrast, D1 agonist administration did not alter cell excitability when applied to spiny cells in slices prepared from the DARPP-32 knockout mice. D2 agonist administration decreased cell excitability in both wild-type and knockout mice. The response produced by combined D1 and D2 agonist stimulation was dependent on the sequence of agonist administration. Thus, the D1 agonist-induced decrease in excitability was reversed to a facilitation of spiking upon subsequent D2 agonist administration. In contrast, D2 agonist applied simultaneously with the D1 agonist only produced a reduction in excitability. This type of D1-dependent modulation was not present in slices from the DARPP-32 knockout mice.

Dopamine (DA) synaptic transmission in the striatal complex occurs via activation of two families of G-protein-linked receptors, the D1- and D2-like DA receptors associated with adenyl cyclase production and inhibition, respectively (Stoof and Keabian, 1981). Activation of striatal DA receptors elicits a multitude of actions, including the modulation of extrinsic and intrinsic synaptic afferents (Calabresi et al., 1987, 2000; Levine et al., 1996; Onn et al., 2000), alteration of paired-pulse response patterns to corticostriatal afferent stimulation (Onn et al., 2000), modulation of electrotonic communication between spiny projection neurons (Cepeda et al., 1989; O’Donnell and Grace, 1993; Onn and Grace, 1994), as well as direct effects on striatal cell membrane excitability (Calabresi et al., 1987; O’Donnell and Grace, 1996).

D1 and D2 receptors show synergistic interactions in which D1 stimulation produces an enabling action for D2-mediated inhibitory effects on striatal cell firing (Walters et al., 1987), on the Na/K membrane pump (Bertorello et al., 1990), and on N-/P-type calcium currents (Bargas et al., 1994). Alternatively, D1 and D2 receptors exhibit opposing actions with respect to the expression of the immediate early gene protein c-Fos, which is induced by either D1 receptor stimulation or by the blockade of D2 receptors (Berretta et al., 1992; Robertson et al., 1992). Although the issue as to whether D1 and D2 receptors are located on distinct subsets of striatal neurons remains a matter of controversy (Surmeier et al., 1992; Aizman et al., 2000), compelling evidence indicates a functional interaction between these two DA receptor subtypes that may occur through receptor-coupled G-proteins that trigger cAMP production (Greengard, 1990; Nishi et al., 1997). A major function of cAMP is the regulation of AMP-dependent protein kinase A, which mediates the phosphorylation of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein 32 kDa; Nishi et al., 1997). Anatomical evidence reveals a close link between D1 receptors and DARPP-32 (Langley et al., 1997); in fact, both exhibit their highest concentrations in the striatal complex (Schalling et al., 1990). Morphologically, DARPP-32 immunoreactivity is enriched in medium-sized spiny neurons that exhibit DA D1 receptors (Surmeier et al., 1992; Langley et al., 1997). DARPP-32 has been subsequently identified in non-D1 containing (Langley et al., 1997) and enkephalin-
containing (Berretta et al., 1992) striatal cells that are thought to contain primarily the D2 receptor subtype (Robertson et al., 1992; Hersch et al., 1995). In contrast to D1 receptors, D2 receptor stimulation dephosphorylates DARPP-32 by activating calcineurin via calcium influx (Greengard, 1990; Nishi et al., 1997). DARPP-32 protein is present in all striatal efferent pathways, including the globus pallidus, the entopeduncular nucleus, and the substantia nigra (Lindskog et al., 1999). Thus, DA modulation of DARPP-32 has the potential to subserve an integrating function with respect to the regulation of striatal outflow via both direct and indirect output pathways (Alexander and Crutcher, 1990; Smith et al., 1998).

A potent tool that has recently been made available is the DARPP-32 knockout mouse in which the gene encoding this protein has been eliminated (Fienberg et al., 1998) resulting in an attenuation of D1-mediated responses (Fienberg et al., 1998; Calabresi et al., 2000). We have therefore used this animal model to examine the temporal interactions of identified striatal neurons to D1 and D2 agonist administration in slices prepared from wild-type versus DARPP-32 knockout mice. In particular, this study is aimed to examine the functional consequences of D1/D2 receptor activation dependent on the time course of the activation of two DA receptor subtypes. Portions of these data have been presented in abstract form (Onn et al., 1996; Grace et al., 1999).

Materials and Methods

Animals and Genotype of Mice. Adult male mice lacking the DARPP-32 gene were generated via gene-targeting in embryonic stem cells using standard techniques. Wild-type and mutant mice were generated by interbreeding of heterozygotes and were typed by southern analysis. The detailed protocol of producing such mutant mice is described elsewhere (Fienberg et al., 1998). All mice used in this study were handled in accordance with the USPHS publication Guide for the Care and Use of Laboratory Animals, and the specific experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and of Drexel University College of Medicine.

Preparation of Mouse Brain Slices. Mice (20–45 g; 3–9 months of age) were deeply anesthetized with chloral hydrate (400 mg/kg b.w., i.p.) before they were perfused transcardially with physiological saline. The physiological saline solution was composed of 124 mM NaCl, 5 mM KCl, 1.2 mM KH2PO4, 2.4 mM CaCl2, 1.3 mM MgSO4, 26 mM NaHCO3, and 10 mM glucose and saturated with 95:5% O2/CO2. The brain was rapidly removed, blocked, and attached with cyanoacrylate adhesive onto the chamber of a Vibratome (Vibratome model series 1000); the chamber was filled with ice-cold physiological saline saturated with 95:5% O2/CO2. Tissue slices (350 μm in thickness) were cut in the horizontal plane parallel to the rhinal fissure and immediately placed into incubation vials containing oxygenated physiological saline solution at room temperature for at least 2 to 3 h before recording. The slices were then placed in the recording chamber maintained at 34–35°C and superfused with continuously oxygenated physiological saline solution at a flow rate of 1 to 2 ml/min under the control of a peristolic pump (model MCP 2500; Haake-Buchler).

Drug Administration. The drugs (±)-SKF 38393 (5–10 μM; Sigma/RBI, Natick, MA) and (−)-quinpirole (5–10 μM; Sigma/RBI) were first dissolved in distilled water at high concentrations to produce stock solutions. Drugs were applied by dissolving them in the oxygenated superfusate-containing reservoir bottle to achieve the specified working concentration. The drug-containing superfusate required approximately 1.5 min to reach the chamber and 3 min to completely replace the medium within the chamber. The time of drug onset described in the results was calculated from the time that the perfusion lines were switched from the control physiological saline to the drug-containing superfusate. The sequential D2 coapplication was typically administered via addition to the reservoir bottle between 10 to 15 min following the onset of D1 agonist administration.

Intracellular Recording and Labeling using Neurobiotin-Filled Microelectrodes. Recording electrodes were constructed from 1-mm o.d. Omegadot (WPI, Sarasota, FL) borosilicate glass tubing using a Flaming-Brown P-80/PC electrode puller and filled with 2% Neurobiotin (dissolved in 3 M potassium acetate; average electrode impedance: 75 to 150 MΩ as measured in situ). Intracellular recordings were performed using a NeuroData 383 intracellular preamplifier, with current injected across a bridge circuit integral to the preamplifier. All membrane potential values were adjusted by subtracting the tip potentials measured at the time point when electrodes were pulled out of cells recorded. The neurobiotin-filled microelectrodes were lowered into the middle region of the dorsal-ventral plane (with the exclusion of nucleus accumbens and the dorsal-most aspects of striatum) under visual control using a stereomicroscope (Nikon SMZ-2B; Melville, NY) by referring to anatomical landmarks, including the anterior commissure, septum, cortical white matter, and internal capsule, with reference to a rat brain stereotaxic atlas (Paxinos and Watson, 1998). After impalement of a cell, 2 to 5 nA constant hyperpolarizing current was applied to achieve a stable impalement; baseline physiological data were then collected at resting membrane potential in the absence of the hyperpolarizing current. After obtaining drug-induced responses, cells were injected with neurobiotin using 0.5 to 1 nA depolarizing current pulses delivered at 1 to 4 Hz (Onn and Grace, 1999). Only one cell per slice was injected to ensure an accurate correlation between the pharmacological responses and cell morphology and location. The slices with neurobiotin-injected cells were then postfixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and cryoprotected in 20% sucrose solution before subjecting to a freeze-thaw procedure to facilitate the penetration of the immunoreagents used for histochemical staining for neurobiotin. The histochemical reactions included a 24-h incubation with avidin-biotinylate complex (Vector, CA) and a 30-min reaction with 3,3’-diaminobenzidine (DAB) (Vector Laboratories, Burlingame, CA), with thorough rinsing with buffer between all steps. The slices were then cleared with 100% dimethyl sulfoxide (DMSO) for 20 min and mounted in DMSO for microscopic examination (Grace and Llinas, 1985). The neurobiotin-filled cells in DMSO-mounted slices were photographed using Kodak Ektasol film (Kodak, Rochester, NY) before being subjected to a subsequent dehydration/defatting process with an alcohol series and xylene to allow mounting of the slices with protex for long-term storage and examination (Onn and Grace, 1994, 1999).

Physiological Measures of the Recorded Cells. The input resistance of the recorded cells was calculated by measuring the membrane voltage deflections produced in response to 100- to 150-ms duration constant hyperpolarizing current pulses (range: 0.2–0.5 nA) delivered across a bridge circuit integral to the preamplifier. Action potential amplitude was measured as the difference between spike threshold and peak of the spike evoked by depolarizing current pulses (150-ms duration) for within-subject comparisons of drug action. When basic spike properties were compared between wild-type and knockout mice, spike amplitude was measured from the resting membrane potential for a between-subject comparison. Our previous studies have shown that the spike amplitude as measured from spike threshold is likely to lead to an underestimate of actual spike amplitude since we reported that a GABAergic conductance located proximal to the spike initiation zone will shift the apparent spike threshold measured at the soma to more depolarized levels (Onn et al., 1994a). Spike threshold was defined as the onset of the fast rising depolarizing phase of the action potentials. Action potential duration was measured across the rising and falling phases of the spike at the membrane voltage corresponding to spike thresh-
old. The amplitude of the after-hyperpolarization following spikes was measured from the voltage corresponding to spike threshold on the falling phase of the spike to the point at which the membrane returned to resting potentials. Rheobase current, defined as the amplitude of current required to generate the first spike discharge, was used to assess the membrane excitability in conjunction with the assessment of spike threshold. Data are presented as mean ± standard error of the mean (S.E.M.) and the unpaired Student’s t test was used for between-subject comparison (i.e., between means of wild-type and knockout mice), whereas the paired Student’s t test was used for within-subject comparison (before and after drug application on the cell tested). Differences were considered to be statistically significant at p < 0.05.

Results

Morphology and Physiological Properties of Striatal Spiny Neurons in Wild-type and DARPP-32 Homozygote Knockout Mice

During recording, all but five striatal neurons (43/48) exhibited physiological properties consistent with the characteristics of the spiny cell class reported in vitro by others (Calabresi et al., 1987, 2000; Cepeda et al., 1993; O’Donnell and Grace, 1993), including a high resting membrane potential, low input resistance, and constant spike firing frequency upon depolarization. The cells did not exhibit fast spike discharge or low-threshold spikes, which are more typically associated with striatal interneurons (Kawaguchi, 1993; Onn and Grace, 1999). The striatal aspiny cells encountered in this study, although infrequent (n = 5), were not readily distinguishable from spiny cells during recording until subsequent observation of their distinct somatodendritic morphology following intracellular staining. Due to differences in their pharmacological responses when compared with spiny cells, the results obtained from recordings of these aspiny cells were not included in this report.

Given that the majority of DA terminals in the striatum form symmetrical synapses onto dendritic spines (Sesack et al., 1994) of the spiny neurons in which most DARPP-32 proteins are located (Greengard, 1990), the morphology of the medium spiny neurons, as revealed by intracellular staining with neurobiotin, was verified for each cell in which pharmacological responses were recorded. Across both genotypes of mice, spiny neurons displayed similar membrane properties with respect to their resting membrane potential, input resistance, spike threshold, spike duration, and spike amplitude for wild-type (n = 23) and homozygote (n = 20) mice, respectively (Table 1). Furthermore, a visual examination of the somatodendritic morphology of these physiologically identified spiny cells indicates that there were no gross differences between wild-type and mutant spiny cells (Fig. 1, A and B; total n = 43) in terms of soma shape, somatodendritic field, number of primary dendrites, etc. and were similar to that previously described for medium spiny neurons in rat striatum (Onn et al., 1994b; Onn and Grace, 1994; 1999). A1 and A2, photomicrographs of a wild-type spiny cell taken before (A1: DMSO mount) and after dehydration/defatting of the tissue (A2: protex mount). Although the spines were readily discernable in the DMSO-mounted preparation (A1), further examination of the same cell after mounting in protex (A2) enhanced the resolution of the spines despite the distortion of the dendritic processes caused by tissue shrinkage due to the dehydration process (Grace and Linas, 1985). B1 and B2, photomicrographs of a spiny cell that was recorded in a homozygote knockout mouse brain slice before (B1) and after (B2) dehydration/defatting. Photomicrographs shown here were taken on 350 μm-thick sections. Calibration bar = 10 μm.

TABLE 1
Passive membrane properties of morphologically identified spiny cells recorded in vitro from mouse striatal slices

Data were expressed as mean ± S.E.M. None of comparisons using a between-subject design reached statistical significance.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Membrane Potential (mV)</th>
<th>Spike Threshold (mV)</th>
<th>Rheobase Current (nA)</th>
<th>Input Resistance (MΩ)</th>
<th>Spike Amplitudea (Peak Potential)</th>
<th>Spike Duration (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (n = 23)</td>
<td>-66.3 ± 1.8</td>
<td>-38.2 ± 2.1</td>
<td>0.58 ± 0.09</td>
<td>59.8 ± 8.9</td>
<td>82.0 ± 8.0</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+18.3 ± 7.3)</td>
<td></td>
</tr>
<tr>
<td>Mutant (n = 20)</td>
<td>-68.1 ± 1.9</td>
<td>-39.4 ± 2.2</td>
<td>0.56 ± 0.09</td>
<td>56.4 ± 6.2</td>
<td>79.7 ± 9.5</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+14.5 ± 8.2)</td>
<td></td>
</tr>
</tbody>
</table>

a Spike amplitude = spike peak voltage – resting membrane potential.
Pharmacological Responses of Spiny Neurons to D1 Agonist (SKF 38393) Administration

Wild-Type Mice. In general, bath application of the D1 agonist SKF 38393 (5–10 μM) to mouse striatal spiny cells (n = 12) caused a decrease in excitability as revealed by a 58.8 ± 13.5% increase in the average rheobase current required to evoke spiking compared with predrug values (D1: 0.89 ± 0.13 nA versus pre-D1: 0.58 ± 0.11 nA; p < 0.006; Table 2). This effect was blocked by the D1 antagonist SCH23390 (5 μM; n = 3). In examining the evoked activity in identified spiny cells (n = 10; 5 for each control group), little accommodation of spike discharge was noted when comparing the first and the second interspike intervals in trains of spikes evoked upon injection of depolarizing pulses <1 nA (Fig. 2A). This firing pattern, which is characteristic of striatal spiny cells in rats (Calabresi et al., 1987, 2000; Cepeda et al., 1993; O'Donnell and Grace, 1996), was not altered following bath application of the D1-agonist SKF 38393 (Fig. 2B).

Spiny neurons that responded to D1 bath application with a decrease in membrane excitability also exhibited a decrease in action potential amplitude by 3.0 ± 2.9 mV, which is consistent with the reported D1-induced attenuation of sodium conductances by 37% (Schiffrann et al., 1995). Although small changes in membrane potential, input resistance, spike threshold, and amplitude were noted; due to the high degree of variability in these parameters when averaged across all cells before and after D1 agonist application in wild-type mouse slices, these D1-induced changes did not reach statistical significance (Table 3). Thus, D1-induced decrease in membrane excitability in wild-type spiny cells was accompanied by a significant increase (58.8 ± 13.5%; p < 0.006) in rheobase current (Fig. 3).

Analysis of the current-voltage relationship in the depolarizing direction revealed an inward rectification that was present in approximately 45% (5/11) of spiny cells tested before D1 agonist application. This rectification was not affected by D1 agonist application. There was no correlation between the presence of inward rectification and the response of the neurons to D1 agonist administration (Fig. 4; top panel). Thus, the presence or absence of inward rectification in the depolarizing direction is independent of the other D1-mediated effects as described above.

Homozygote DARPP32-Knockout Mice. As observed in spiny cells from wild-type mice, spiny cells in slices prepared from knockout mice responded to D1 agonist administration with a hyperpolarization of their membrane potentials (n = 7/12) that was similar in magnitude to that observed in wild-type neurons; nonetheless, the difference did not reach statistical significance in either case (knockout: hyperpolarized by 1.3 ± 1.1 mV versus wild-type: 2.7 ± 1.5 mV; Table 3). Similarly, there were no significant differences noted in the input resistance (knockout: decreased by 3.0 ± 8.8 MΩ versus wild-type: increased by 25.3 ± 12.2 MΩ; p = 0.71; N.S.) in the spike amplitude (knockout: decreased by 0.9 ± 2.2 mV versus wild-type: decreased by 5.2 ± 1.7 mV; p = 0.29; N.S.) between wild-type and mutant spiny cells. Nevertheless, the magnitude of D1-induced changes in the rheobase current (knockout: 1.5 ± 4.1% versus wild-type: 58.8 ± 13.5%; *p = 0.0012, unpaired Student’s t test) were significantly smaller in mutant spiny cells than those observed in wild-type spiny cells (Table 3; Fig. 3). In summary, mutant spiny cells typically responded to D1-like agonist application with negligible changes in resting membrane potential, input resistance, spike threshold and amplitude, and rheobase current. The inward rectification in the depolarizing direction that was present in three of eight mutant spiny cells was not altered by D1 agonist administration (Fig. 4; bottom panel). In fact, the D1 agonist was found to enhance this property in three other mutant spiny cells that did not display this rectification before D1 agonist application.

Pharmacological Responses of Spiny Neurons to Combined D1 and D2 Agonist Administration

Wild-Type. Application of the D2 agonist quinpirole caused a decrease in cell excitability (n = 12) independent of whether it was administered alone (n = 6) or in combination with the D1 agonist (n = 6). Although D2 agonist administration did not cause consistent changes in membrane potentials (range = 10 to +11 mV) and resistances (–7 to + 5 MΩ), it did result in an increase in spike threshold by 5 to 15 mV (average = +7.8 ± 3.7 mV) (Fig. 5; Table 4) when administered alone (n = 6; +7.6 ± 3.8 mV) or with the D1 agonist (n = 6; +8.1 ± 3.7 mV); thus data from both groups were pooled. The large variability indicates that in either situation D2 inhibitory action was rather varied, e.g., from mild insignificant excitation/inhibition to a potent inhibition of membrane excitability, and this may be a reflection of D2-mediated actions on both presynaptic and postsynaptic sites (O’Donnell and Grace, 1994, 1996). Thus, as noted above the changes in membrane potentials were highly variable and did not approach statistical significance; however, changes in spike threshold and amplitude did occur consistently and were independent of the change in membrane polarization. This decrease in membrane excitability was reflected by an average 47% increase in rheobase current (45 ± 39 for D2 alone; 51 ± 47% for D1 and D2 combined; Table 4). Simultaneous application of D1/D2 agonists (Fig. 5B) tended to cause a greater amplitude of membrane depolarization elicited by a given current pulse amplitude when compared with the agonist administered alone (Fig. 5A). Thus, D2 agonists when applied alone or simultaneously with D1 agonists produced a reduction in membrane excitability in wild-type spiny cells.

In slices (n = 9) in which administration of SKF38393 resulted in a decrease in membrane excitability, subsequent coadministration of quinpirole (5–10 μM) at 10 to 15 min

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**Table 2**

Comparison of rheobase currents in spiny cells following SKF38393 and subsequent coapplication of D2 agonist in wild-type versus mutant mouse slices.

<table>
<thead>
<tr>
<th></th>
<th>Predrug</th>
<th>SKF38393 (5 min)</th>
<th>+ Quinpirol (5 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.58 ± 0.08</td>
<td>0.89 ± 0.13**</td>
<td>0.523 ± 0.135**</td>
</tr>
<tr>
<td>Spiny Cells</td>
<td>(n = 12)</td>
<td>(n = 12)</td>
<td>(n = 12)</td>
</tr>
<tr>
<td>Mutant</td>
<td>0.56 ± 0.09</td>
<td>0.58 ± 0.11</td>
<td>0.460 ± 0.150*</td>
</tr>
<tr>
<td>Spiny Cells</td>
<td>(n = 6)</td>
<td>(n = 6)</td>
<td>(n = 6)</td>
</tr>
</tbody>
</table>

* Paired Student’s t test, P < 0.05 compared to SKF38393 in a within-subject design.
** Paired Student’s t test, P < 0.005 compared to predrug current in a within-subject design.
* Paired Student’s t test, P < 0.006 compared to predrug current in a within-subject design.
following SKF 38393 was found to restore the excitability of these cells to baseline levels \((n = 6/9)\). This was reflected by a D2-mediated restoration of spike threshold \((-5.1 \pm 2.4 \text{ mV})\) and threshold current \((-63 \pm 22\% \text{ from the post-D1 levels})\) to the initial predrug levels (Table 4; Fig. 6A1–3). The average rheobase current observed following subsequent D2 agonist coapplication \((-0.9 \pm 2.2 \text{ nA})\) was nearly identical with the initial \((-0.89 \pm 0.13 \text{ nA}; \ * \text{ N.S.})\). Furthermore, the reduction in spike amplitude produced by D1 agonist application \((-3.0 \pm 2.9 \text{ mV})\) was restored to initial (predrug) levels following subsequent D2 agonist administration \((-2.7 \pm 3.1 \text{ mV relative to post-D1 level}; \ Table 4)\).

### Mutant Spiny Cells

The D2-induced reduction in membrane excitability in wild-type spiny cells, expressed as an increase in spike threshold and rheobase current (see above), was preserved in mutant spiny cells \((n = 8)\). Furthermore, the decrease in spike threshold and rheobase current observed following subsequent D2 agonist administration \((-7.1 \pm 2.3 \text{ mV and threshold current } -42 \pm 22\%)\) Moreover, although D1 agonists failed to alter the excitability of cells recorded in slices from DARPP-32 knockout mice (see above), subsequent coadministration of the D2 agonist quinpirole also did not increase cell excitability \((n = 5)\), as it did in wild-type spiny cells. Thus, the average rheobase current

<table>
<thead>
<tr>
<th>Membrane Potential</th>
<th>Input Resistance (MΩ)</th>
<th>Spike Threshold (mV)</th>
<th>Spike Amplitude (mV)</th>
<th>Rheobase Current (nA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type ((n = 8))</td>
<td>-2.7 ± 1.5</td>
<td>+25.3 ± 12.2</td>
<td>+5.2 ± 1.7</td>
<td>-3.0 ± 2.9</td>
</tr>
<tr>
<td>Mutant ((n = 8))</td>
<td>-1.3 ± 1.1</td>
<td>-3.0 ± 8.8</td>
<td>+2.4 ± 1.9</td>
<td>-0.9 ± 2.2</td>
</tr>
</tbody>
</table>

* Spike amplitude = spike peak - spike threshold.

* Unpaired Student’s *t* test, \(P = 0.0012\) compared to wild-type spiny cells using a between-subject comparison. Other comparisons did not reach statistical significance.

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**Table 3**

D1 agonist-induced changes in passive membrane properties of spiny cells recorded in vitro from wild-type and mutant mouse slices

Data were expressed as mean ± S.E.M.
during D2 agonist coapplication was found to decrease by 20% to 0.46 ± 0.15 nA when compared with that observed during D1 agonist application (0.58 ± 0.11 nA) or with respect to the initial predrug level (0.56 ± 0.09 nA; Table 2).

**Discussion**

Of the 48 neurons labeled during recordings, data were analyzed from 43 neurons that were medium spiny in somatodendritic morphology. All of these neurons in normal mouse striatum were similar in somatodendritic morphology to spiny neurons labeled intracellularly in vitro in rat striatum or accumbens (Calabresi et al., 1987, 2000; Cepeda et al., 1993; O'Donnell and Grace, 1993). Furthermore, the basic membrane properties of knockout spiny neurons were similar to those of spiny neurons recorded in wild-type mouse slices. In contrast, in spiny neurons in the DARPP-32 knockout mice, stimulation of dopamine D1 receptors failed to produce the increase in spike threshold and threshold current observed in the wild-type. One apparent consequence of this loss of D1-mediated actions is that subsequent stimulation of D2 receptors in the knockout mouse slices produced qualitatively different responses from those typically observed in wild-type spiny neurons. Whether this modification is due to the loss of D1 modulation or instead is due to developmental and/or compensatory changes is unclear. Nonetheless, these pharmacological data reveal a complex alteration in dopamine transmission in the DARPP-32 knockout mouse striatum.

**Dopamine Modulation of Membrane Excitability in Spiny Neurons.** Averaged across the population of medium spiny cells tested, D1 or D2 agonist administration did not significantly alter membrane potential. Nonetheless, administration of the D1 agonist SKF 38393 caused a 3 to 11 mV

![Fig. 3. D1-induced changes in spike threshold, spike amplitude, and response to depolarization were compared in spiny cells recorded in wild-type (A) and mutant (B) mouse brain slices. A1 to 3, overlay of voltage traces taken before and after (arrowheads) D1 agonist administration. D1 agonist administration caused a hyperpolarization of the membrane and increased the amplitude of membrane depolarization produced in response to the same amplitude of depolarizing current (A2). Nonetheless, because of the increase in spike threshold (A3: predrug threshold = −47 mV; after D1 agonist = −39 mV), higher amplitudes of current injection were required to elicit spike discharge after D1 agonist administration. In contrast, in the DARPP-32 knockout mice D1 agonist administration did not alter the resting membrane potential (B1: −70 mV before and after D1), the membrane voltage deflection produced by depolarization current injection (B2) or the spike threshold (B3: −50 mV before and after D1). Traces in B1 to 3 marked by arrows were taken after D1 application.](image-url)
hyperpolarization in 62% of the cells tested, whereas administration of the D2 agonist quinpirole caused depolarization in 56% of the cells tested. Others have shown that DA acting via D1 receptor activation results in membrane hyperpolarization in striatal cells (Uchimura et al., 1986; Calabresi et al., 1987). In contrast, application of DA at higher concentrations (i.e., 100–400 μM) appears to depolarize striatal spiny neurons, presumably by a D2 receptor-induced decrease in potassium conductances (Uchimura et al., 1986). In the present study, we chose to use DA agonists applied at moderate doses (5–10 μM), as derived from the literature (Calabresi et al., 1987, 2000; Cepeda et al., 1993, 1995; O’Donnell and Grace, 1996). Thus, activation of either dopamine receptor subtype reduced membrane excitability in the wild-type spiny cells, as demonstrated by an increase in spike threshold and threshold current required to elicit spike discharge, independent of its effect on membrane potential.

Eighty percent of spiny cells recorded in slices prepared from wild-type mouse responded to the D1 agonist SKF 38393, with an increase in spike threshold and rheobase current, resulting in a suppression of spike discharge and a decrease in spike amplitude. These D1-induced changes are consistent with an alteration in sodium conductances. Dopamine is known to act on the slowly inactivating Na+/K+ conductances to modulate the threshold current for evoking spikes in striatal neurons (Calabresi et al., 1987; Cepeda et al., 1995; Schiffmann et al., 1994). This is believed to occur via the phosphorylation of sodium channels by the cAMP-dependent protein kinase A, resulting in a reduction in the open probability of sodium channels (Cepeda et al., 1995; Schiffmann et al., 1994). This is consistent with the apparent absence of D1-induced changes in spiny neurons recorded in knockout mouse slices in which the DARPP-32 proteins were absent. D1 agonists have also been reported to decrease N- and P-type while increasing L-type calcium currents in striatal cells via the DARPP-32 signal transduction pathway.

**Fig. 4.** Effects of the D1 agonist SKF38393 on the passive membrane properties of wild-type (top panels) and DARPP-32 knockout (bottom panels) mouse brain slices. Top panel (left), voltage traces produced in response to a series of current pulses observed in the morphologically identified spiny cell before application of the D1 agonist SKF38393. In response to depolarizing current pulses, a slow depolarization preceding spike discharge [threshold (TH) = −47 mV was noted in this cell]. Top panel (center): membrane voltage responses observed in the same cell following 5-min bath application of the D1 agonist SKF38393 (11 μM). Application of this D1 agonist resulted in hyperpolarization of the membrane of this cell by 8 mV and increased the threshold for spike discharge (predrug = −47 mV; after SKF38393 = −39 mV). Top panel (right), a current-voltage plot was constructed from the traces shown in A1 and A2 by plotting the voltage deflections from the resting membrane potential (RMP = −75 mV) against the amplitude of current injected. The regression line of the I-V plot in the hyperpolarizing direction reveals a predrug input resistance of 70 MΩ (circles) versus 81 MΩ following D1 bath application (squares). In this cell, the absence of a deviation of the regression from linearity is consistent with the lack of a prominent inward rectification in this cell. Bottom panels, membrane voltage responses observed in an identified spiny cell recorded in knockout mouse slices before (left panel) and after (center panel) D1 agonist application. Administration of SKF38393 failed to produce a significant hyperpolarization of the membrane of this neuron (predrug = −80 mV versus D1 agonist = −78 mV), nor did it alter the spike threshold (predrug = −42 mV versus D1 agonist = −43 mV). The current-voltage plots (right panel) constructed from these responses revealed no alteration in input resistance (predrug (circles) = 42 MΩ; D1 agonist (squares) = 44 MΩ). The membrane rectification, observed as a deviation from linearity in both the hyperpolarizing and depolarizing directions, was not altered by D1 agonist administration.
L-Type calcium channels appear to be activated by D1 receptors only when the cell membrane is depolarized to \(-50 \text{ mV}\) or above (Hernandez-Lopez et al., 1997), however, with the result being an excitatory response to D1 receptor activation. This D1-induced excitation at depolarized membrane potentials is proposed to result from coactivation of N-methyl-D-aspartate receptors (Cepeda et al., 1993); thus, it is not likely to account for D1-induced excitation at depolarized membrane potentials. The effect of the D2 agonist quinpirole, administered alone or in conjunction with the D1 agonist, on the evoked action potential discharge in wild-type spiny cells is illustrated in Fig. 5. A1 to 3, overlay of voltage traces taken before and after (arrows) quinpirole application. Following quinpirole administration, depolarizing current injection produced comparatively greater levels of membrane depolarization (A1 and A2) as well as causing an increase in spike threshold (A3: predrug = -34 mV; quinpirole = -15 mV) compared with control conditions. In addition, there was a decrease in spike amplitude by 19 mV (relative to spike threshold) following quinpirole. B1 and 2, overlays of voltage/current traces taken before and after (arrows) combined application of D1/D2 agonists. Following coadministration of SKF 38393 (10 \(\mu\)M) and quinpirole (10 \(\mu\)M), there was a depolarization of the membrane from -70 to -53 mV. Furthermore, although a greater amount of depolarizing current injection produced a larger depolarization of the membrane (B1: predrug = 18 mV; D1/D2 = 25 mV for a 0.26-nA current injection amplitude), there was also a substantial increase in spike threshold (B2 from -40 to -17.5 mV). In response to the same amplitude of current injection, simultaneous administration of D1 and D2 agonists produced more voltage depolarization (B1) and a more depolarized spike threshold (B2) as compared with that measured following bath application of the D1 agonist alone or D2 agonist alone.

| Table 4: Comparison of membrane potential, threshold voltage, rheobase current, and spike amplitude in wild-type spiny cells following bath application of SKF38393 and quinpirole |
|---|---|---|---|
| Membrane Potential | Spike Threshold | Rheobase Current | Spike Amplitude* |
| SKF38393 | mV | mV | % | mV |
| Quinpirole | -2.7 \(\pm\) 1.5 | +5.2 \(\pm\) 1.7 | +58.8 \(\pm\) 13.5 | -3.0 \(\pm\) 2.9 |
| D1 + D2 | +1.2 \(\pm\) 9.0 | +7.6 \(\pm\) 3.8 | +45.0 \(\pm\) 39.1 | -11.4 \(\pm\) 10.7* |
| Simultaneously | +3.1 \(\pm\) 4.3 | +8.1 \(\pm\) 3.7 | +51.0 \(\pm\) 47.0 | -10.4 \(\pm\) 13.0 |
| D1 -> D2 Sequentially | +4.3 \(\pm\) 2.5 | -5.1 \(\pm\) 2.4** | -63.0 \(\pm\) 22.0 | +2.7 \(\pm\) 3.1** |

* Spike amplitude = spike peak - spike threshold
* Paired Student’s t test \((P < 0.05)\) compared to SKF38393 for a within-subject comparison.
** Paired Student’s t test \((P < 0.05)\) compared to D1 + D2 simultaneously for a within-subject comparison.
increases in spike threshold and rheobase current observed in the present study.

Seventy percent of cells recorded in this study responded to either the D1-like and/or the D2-like agonist, suggesting that striatal spiny cells may exhibit either a high degree of D1 and D2 receptor colocalization (Surmeier et al., 1992; Hersch et al., 1995; Aizman et al., 2000) or that these agonists exert influences on common sets of interneurons that innervate the spiny cells (Lindskog et al., 1999). At least part of the D1/D2-induced response is mediated postsynaptically since it suppressed spike discharge evoked by depolarizing current pulses injected into the recorded cells. Furthermore, other studies have demonstrated that this D1-induced response persists in calcium-free or in tetrodotoxin-containing (Calabresi et al., 1987; Cepeda et al., 1995) superfusion buffer in which synaptic transmission is presumably blocked.

It appears that there is some residual D1 effect in the knockout spiny neuron that somehow modified the subsequent administration of the D2 agonist to enable a small excitatory action; nonetheless, the response was substantially less than that observed with D1 agonist administration in the wild-type neurons. In addition, the finding that the D2-induced reduction of membrane excitability persisted in the knockout spiny neurons suggests that signal transduction pathways other than the PKA-mediated DARPP-32 pathway may be involved in these responses.

**Functional Implications of D1/D2 Receptor Interaction.** Our data indicate that simultaneous stimulation of D1 and D2 receptors causes a potentiated inhibition of striatal neuron activity, whereas D2 stimulation will reverse D1-mediated inhibition if applied at a later time. This arrangement may have functional implications with respect to the temporal regulation of striatal neurons by DA. Studies have shown that D1 and D2 receptors are located both intrasynaptically as well as along the extrasynaptic membrane of the neuron, with relatively higher numbers of D1 receptors located extrasynaptically and D2 receptors concentrated within the synaptic cleft (Sesack et al., 1994; Smiley et al., 1994; Khan et al., 1998). Therefore, one would expect that a moderate activation of the DA system should lead to sufficient spike-dependent phasic DA release.

![Fig. 6](image_url)

**Wildtype Spiny Cell**

**Mutant Spiny Cell**

A1. D2 added to D1

A2. pre-drug TH = -30 mV

A3. D2 added to D1

B1. D2 added to D1

B2. D1 alone

B3. D2 added to D1

Fig. 6. In contrast to the effects produced by simultaneous administration of SKF 38393 and quinpirole, sequential administration of SKF 38393 followed by coadministration of quinpirole produced substantially different responses in wild-type but not mutant mouse slices. A1 to 3, in wild-type mouse slices in which SKF 38393 (10 μM) had been infused for at least 10 min, subsequent addition of quinpirole (10 μM) caused depolarization of the membrane potential (A1: D1 alone = -58 mV; D1 + D2 = -40 mV). In addition, quinpirole caused a decrease in spike threshold (A1: D1 + D2 = -30 mV versus A3: D1 alone = -22 mV) to approximately the same membrane potential spike threshold values recorded before D1 agonist administration (A2: predrug = -30 mV). Thus, in this cell, the rheobase current before the drugs, after D1 and after D2 added to D1 is 0.32 nA (A2), 0.42 nA (A3), and 0.15 nA (A1), respectively. B1 to 3, in contrast, in the knockout mouse slices, exposure to SKF 38393 (10 μM) failed to produce the same type of changes in membrane excitability as those observed in wild-type slices. Instead, there was no change in resting membrane potential, and there was a small increase in spike threshold (D1 alone = -54 mV; D1 + D2 = -47 mV).
(Grace, 1991) to stimulate the intrasynaptic D2 receptors, thus leading to moderate inhibition of striatal neuron excitability. On the other hand, if the DA system is subjected to a powerful drive, such as during burst firing of DA neurons in response to reward-associated stimuli (Schultz, 1992; Wickens et al., 1996; Gonon, 1997), the resultant massive phasic DA release (Grace, 1991) should be of sufficient magnitude to stimulate both D1 and D2 receptors within the synapse, as well as diffuse from the synapse to stimulate the extrasynaptic receptors. Drawing from our data, such simultaneous stimulation should lead to a potentiated inhibitory effect of DA on the striatal neuron. If DA system stimulation is maintained, however, the tonic extrasynaptic DA pool would provide baseline stimulation of the extrasynaptic D1 receptors. As a result, the subsequent phasic DA release within the synapse onto D2 receptors would instead produce a disinhibitory outcome. Indeed, such an arrangement may account for the facilitatory effects of DA that were produced in response to high frequency stimulation of DA fibers in vivo (Gonon, 1997). Thus, according to this model, the system would appear to be designed to attenuate phasic DA responses under conditions in which the DA system is being tonically overdriven.

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References

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