Expression of a dopamine D2 receptor-activated K+ channel on identified striatopallidal and striatonigral neurons

BARBARA L. WASZCZAK*, LYNN P. MARTIN, GABRIELA J. GREIF†, AND JONATHAN E. FREEDMAN

Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115

ABSTRACT One view of the efferent circuitry of the basal ganglia holds that dopamine D1 and D2 receptors are segregated to striatogniral and striatopallidal neurons, respectively. The present studies investigated whether functional D2-like receptors are, in fact, restricted to striatopallidal neurons. Single, freshly dissociated cells from rat striatum were identified as either striatogniral or striatopallidal projection neurons by fluorescence retrograde labeling. By using cell-attached patch-clamp recordings, neurons of each efferent group were evaluated for the presence of a D2-like receptor-activated 85-pS K+ channel as a measure of receptor function. We now report the presence of this D2 receptor-mediated response on both striatal efferent populations, but we observed an approximately 2-fold higher likelihood of encountering the channel on pallidal- versus nigral-projecting neurons. The channel’s conductance properties appeared identical in both groups of neurons, but there was a significantly greater open probability for channels detected on striatopallidal neurons. These results indicate that functional D2 receptors are not segregated to striatopallidal neurons, but may be expressed in a higher proportion of, or at a higher density and/or efficiency of coupling on, pallidal- versus nigral-projecting striatal efferents.

Medium spiny neurons of the corpus striatum project to the substantia nigra and to the globus pallidus. The current functional model of the basal ganglia hypothesizes that dopamine exerts opposite physiological effects on striatogniral and striatopallidal efferent populations (excitatory and inhibitory, respectively) (1), and that these opposing effects of dopamine are mediated by a segregated and specific expression of D1 and D2 dopamine receptors, respectively, on the two efferent cell groups (2). This model has been supported by the findings of numerous investigators using varied approaches. However, more recent reports have suggested that a significant amount of D1 and D2 receptor colocalization may occur on striatal projection neurons (3–8). This issue is an important one because if coexpression of the two receptors on striatal efferents is common, this would imply that dopamine does not separately and differentially regulate striatogniral and striatopallidal output in the manner predicted by the basal ganglia model. The model would then need to be revised to accommodate the possibility that the two receptors interact on at least a subpopulation of striatal neurons to regulate the activities of both efferent cell groups.

The controversy surrounding the degree of D1 and D2 receptor colocalization stems in part from limitations of the previously used techniques for both detecting the receptors and identifying the two projection populations. A majority of the previous studies have measured the cellular expression or distribution of D1 and D2 receptor mRNAs (2–5, 9–11), or the binding of selective ligands (8) or antibodies to the receptors (6, 12, 13). The two efferent populations have been distinguished either by retrograde labeling or by their expression of projection-related peptide markers (mRNAs for substance P or dynorphin for striatogniral neurons and enkephalin for striatopallidal neurons). These studies yielded vastly different estimates of the extent of receptor colocalization ranging from virtually none (2, 10, 12, 13) to as much as 50–60% of striatal neurons expressing both receptors (3, 6, 7, 11). Part of the discrepancy in these estimates may be a result of the methods for distinguishing the two neuronal populations. For instance, studies relying on enkephalin or substance P mRNAs for identifying the two projections might overestimate the degree of D1/D2 colocalization since these peptides have slightly overlapping expressions in the two efferent groups (5, 14). Beyond this, the means of detection of the receptors has been controversial. It has been argued that detection of a receptor mRNA, especially if in low abundance, is not necessarily indicative of physiologically significant amounts of expressed receptor protein (15). Conversely, detection of receptor protein using subtype-selective antibodies may be subject to criticisms of antibody selectivity and sensitivity. Finally, immunohistochemical detection of receptor protein may not necessarily correlate with the presence of functional receptors. A more definitive way to resolve this controversy may be by measurements of specific dopamine receptor-mediated functional responses on isolated striatal neurons, which have been identified by retrograde labeling from their projection sites.

We have identified a specific D2-mediated response of striatal neurons that may be useful as a means of detecting the expression of functional D2 receptors on identified neural populations. The response is the activation by quinpirole, a selective agonist at the D2-like group of dopamine receptors (16, 17), of an inwardly rectifying 85-pS K+ channel on freshly dissociated striatal neurons studied by cell-attached patch-clamp electrophysiology (18). Openings of the 85-pS channel occur in a membrane-delimited manner (19) and have an absolute dependence on the presence of dopamine or a D2-like agonist in the cell-attached patch pipette, but are never observed in the absence of drug, nor when a D2 antagonist is included with quinpirole (18, 20). In addition, this channel is not activated by D1-like receptor agonists, nor is its activation by dopamine or D2-like agonists blocked by the presence of a D1 antagonist in the patch pipette (unpublished observations). The channel has been shown to be restricted in its expression to striatal neurons with diameters of 10–20 μm and with morphological features similar to a subpopulation of dissociated striatal neurons that stain positively for neuron-specific enolase and γ-aminobutyric acid (GABA) immunoreactivity.
These features suggest that striatal neurons that exhibit 85-pS K⁺ channel openings in the presence of quinpirole express functional D2-like receptors and that these neurons might include GABAergic medium spiny projection neurons. We therefore have examined the frequency of observing this channel on dissociated striatal neurons that have been labeled retrogradely using fluorescent latex microspheres from projection sites in either the globus pallidus or substantia nigra pars reticulata.

METHODS

Retrograde Labeling. Male Sprague–Dawley rats (Taconic Laboratories) between 28 and 30 days postpartum were anesthetized with ketamine (80 mg/kg) and xylazine (12 mg/kg), i.p., and then positioned in a stereotaxic apparatus. Red or green fluorescent latex microspheres (Molecular Probes) were injected into either the substantia nigra pars reticulata or globus pallidus over 10 min by using a 1-μl Hamilton syringe controlled by a Kopf microinjection apparatus attached to the stereotaxic device. In some experiments, both structures were injected, each with a different color microsphere. The needle was allowed to remain in place for 10 min and then withdrawn slowly. For substantia nigra, 0.5-μl fluorescent microspheres were injected at 2.0 mm L, 2.7 mm A (to the lamina dura suture), and 8.0 mm V. For globus pallidus, 0.4 μl was injected at an 18° forward-directed angle (to avoid passing the needle through the caudal part of the striatum) at 2.7 mm L, −3.9 mm A (from bregma), and 6.9 mm V. Animals were sacrificed 4–6 days after surgery, the injection field and lack of spread into the caudate-putamen were verified histologically, and striatal projection neurons, and then under phase-contrast optics to exclude globus pallidus from dissections of the caudate-putamen. Cells were viewed with a Nikon Diaphot microscope by first using a fluorescence filter set to locate retrogradely labeled neurons, and then under phase-contrast optics to determine whether the labeled cell appeared phase-bright and undamaged. Fluorescent latex microspheres were illuminated with a mercury lamp and viewed with a fluorescein-type filter set for yellow-green microspheres (420 ± 10 nm excitation wavelength, 505 nm dichroic mirror, 535 ± 20 nm emission filter) or an allophycocyanin-type filter set for red microspheres (590 ± 22 excitation, 620 dichroic mirror, 660 ± 16 emission). Only cells that appeared phase-bright and contained fluorescent beads were used in these experiments. We verified that labeled cells contained only beads fluorescing at one wavelength or the other.

Patch-Clamp Electrophysiology. Striatal neurons were freshly dissociated as previously described (18) from rats injected with fluorescent latex microspheres. Care was taken to exclude globus pallidus from dissections of the caudate-putamen. Cells were viewed with a Nikon Diaphot microscope by first using a fluorescence filter set to locate retrogradely labeled neurons, and then under phase-contrast optics to determine whether the labeled cell appeared phase-bright and undamaged. Fluorescent latex microspheres were illuminated with a mercury lamp and viewed with a fluorescein-type filter set for yellow-green microspheres (420 ± 10 nm excitation wavelength, 505 nm dichroic mirror, 535 ± 20 nm emission filter) or an allophycocyanin-type filter set for red microspheres (590 ± 22 excitation, 620 dichroic mirror, 660 ± 16 emission). Only cells that appeared phase-bright and contained fluorescent beads were used in these experiments. We verified that labeled cells contained only beads fluorescing at one wavelength or the other.

Patch-clamp recordings from retrogradely labeled neurons were performed in the cell-attached configuration, as described previously (18), with 10 μM quinpirole in the patch pipette. Electrodes had tip diameters ranging from 0.7 to 1 μm and resistances of about 2 MΩ when filled with solution. Criteria for satisfactory recordings were as described previously (18). Currents were measured by using an Axopatch 1D system interfaced with a 386-based computer and analyzed using PC-LAMP software (Axon Instruments, Foster City, CA). After filtering at 2 kHz low pass, data were acquired to the computer at 100 μsec/poin and viewed on-line with an analog oscilloscope. Single K⁺ channel currents were measured as inward currents; the 85-pS channel was distinguished from other channels by its unitary conductance and the voltage insensitivity of its activation (18). Membrane potential cannot be measured directly in cell-attached recordings and so was expressed as resting potential plus the pipette potential multiplied by −1. The number of active channels per patch was estimated from simultaneous openings detected over an observation period of approximately 10 min and from the number of peaks in all-points amplitude histograms, which were generated from digital records of at least 25,600 points. Fractional open and closed times were determined from areas under the peaks of the amplitude histograms. The fractional open probability, $P_{o}$, was defined as $1 - N_{c}$, where $N$ was the number of channels in the patch and the closed probability, $P_{c}$, was the fraction of time no channel current was passed. Statistical comparisons of cell populations were performed by using a Student's t test.

RESULTS

Cell-attached patch-clamp recordings were made from a total of 24 retrogradely labeled striatopallidal neurons and 47 striatonigral neurons with 10 μM quinpirole in the patch pipette. Of these, 9 pallidal-projecting neurons and 8 nigral-projecting neurons exhibited 85-pS channel openings after seal formation (Table 1). Examples of recordings of the channel on the two cell groups are shown in Fig. 1. The presence of the channel in these recordings indicates the presence of the D2 receptor-mediated response on both striatal efferent populations, although there was an approximately 2-fold greater occurrence of the channel on pallidal- versus nigral-projecting neurons.

To determine whether heterogeneous forms of the D2-coupled K⁺ channel might exist in the two cell groups, single-channel conductances were assessed from current–voltage relationships. Current–voltage plots for the 85-pS channel on striatopallidal and striatonigral neurons were almost superimposable over a range of voltages from 40 mV hyperpolarized to 40 mV depolarized, relative to resting potential (Fig. 2). There similarly were no apparent differences in the extrapolated reversal potentials, nor in the apparent resting potentials of the two cell groups, as inferred from the reversal potential. The uniformity of the channel's conductance properties suggests that the 85-pS K⁺ channel itself, as expressed on the two cell populations, is likely the same molecular entity.

As previously reported (18), we often observed multiple 85-pS channel openings in the same patch. Conceivably, a higher density of D2 receptor expression might correlate with an increase in the numbers of channels observed per patch, so we evaluated recordings from the two cell populations to assess whether there was a difference in this parameter. Nigral-projecting neurons had a greater likelihood of a single channel per patch (five of eight patches) and a lower likelihood of three or more channels per patch (one of eight patches) than did pallidal-projecting neurons (three of nine patches with a single channel per patch and three of nine patches with three or more channels per patch; examples in Fig. 3A). However, we were not able to make an accurate statistical comparison of the numbers of channels per patch between the two cell populations since such comparisons are reliant on a uniform pipette tip diameter and a uniform membrane surface area within the patch, and our pipette tip diameters were variable over a range of from 0.7 to 1 μm.

Alternatively, we have approached the question of possible differences in D2 receptor density and/or functionality by comparing the fractional open probability ($P_{o}$) of individual channels from the two cell populations, as assessed from all-points amplitude histograms. This parameter is independent of the size of the membrane patch. The 85-pS K⁺ channels

Table 1. Frequency of expression of the 85-pS K⁺ channel on identified striatal projection neurons

<table>
<thead>
<tr>
<th>Neurons</th>
<th>No. of cells tested</th>
<th>No. of cells with channel (%)</th>
</tr>
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<tbody>
<tr>
<td>Striatonigral</td>
<td>47</td>
<td>8 (17%)</td>
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<tr>
<td>Striatopallidal</td>
<td>24</td>
<td>9 (38%)</td>
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encountered on striatopallidal cells had a significantly greater mean $P_o$ than those observed on striatonigral neurons (0.76 versus 0.48, $P < 0.01$; Fig. 3B), consistent with there being either a higher density of D2 receptors capable of accessing and opening individual channels, or a greater efficiency of receptor-channel coupling, on pallidal- versus nigrally-projecting neurons.

**DISCUSSION**

Using a combination of retrograde labeling and cell-attached patch-clamp recordings of freshly isolated striatal neurons, we have been able to identify cells that express a specific D2 receptor-mediated response, i.e., activation by quinpirole of an 85-pS $K_1$ channel. The major finding of this study is that functional D2-like receptors, as assessed by the activation of this channel, are not segregated exclusively to striatopallidal efferents, but are also present on a significant subpopulation of striatonigral neurons. Since we have measured an electrophysiological response associated with D2 receptor activation, we were able to avoid the possibility of detecting functionally inactive receptors. Alternative approaches without a functional endpoint, such as measuring D2 mRNA levels, ligand binding, or receptor immunoreactivity, might not give accurate estimates of the proportions of neurons in each efferent population that express physiologically active D2 receptors. Since the cell dissociation procedure in our studies requires only a few hours, it is relatively improbable that these channel effects were an artifact of in vitro conditions. Similarly, because the cells were isolated, and the D2 agonist was present only in the patch pipette, we can attribute agonist effects directly to the cells recorded from, and to receptors present within, the isolated patch of membrane. Although the 85-pS $K_1$ channel may be only one of a number of ion channels on striatal neurons that are modulated by D2 receptors, this channel has been shown in recordings from several hundred neurons to be coupled specifically to D2-like receptors, and so its activation can be used as a functional marker of D2-like receptor expression on single striatal neurons (18–20).

The proportions of striatopallidal and striatonigral neurons that express functional D2-like receptors might be estimated by extrapolation from our data, taking into account certain limitations of the technique. We found an approximately 2-fold greater likelihood of encountering the channel in patches on striatopallidal (38%) than striatonigral neurons (17%). A failure to observe 85-pS channel openings on the remaining neurons does not, however, rule out the presence of D2 receptors or the channel on these neurons, but only indicates that none were captured in the small patch of membrane under the pipette tip. Actual percentages of neurons of both projection types that express the D2 receptor-coupled channel are likely to be greater than the percentages detected by this method. For instance, although we encountered the D2-coupled channel in only 38% of patches on pallidal-projecting cells, it has been reported that virtually all enkephalin-containing (thus striatopallidal) neurons express D2 receptor mRNA (9). It then might be expected that although we observed the channel on 17% of striatonigral

**FIG. 1.** Cell-attached patch-clamp recordings of single 85-pS D2 dopamine receptor-activated $K_1$ channels on identified striatopallidal (A) and striatonigral (B) neurons. Both records are at resting membrane potential.

**FIG. 2.** Current–voltage relationships for D2 dopamine receptor-activated $K_1$ channels on identified striatopallidal and striatonigral neurons. Voltage is expressed relative to resting membrane potential (RMP). The lines were fitted by linear regression, with slopes corresponding to 85 pS. Data are expressed as mean ± SD for two to four cells at each point.

**FIG. 3.** Comparison of fractional open probabilities of the D2 dopamine receptor-activated $K_1$ channel on identified striatonigral and striatopallidal neurons. (A) Examples of all-points amplitude histograms of a striatonigral cell with one active channel in the patch (Left) and a striatopallidal cell with three active channels in the patch (Right). In each case, the relative amplitude of the peak at 0 pA is proportional to the fraction of time spent in the closed state. (B) Distribution of fractional open probability ($P_o$) values for striatonigral and striatopallidal neurons. Each point represents the value for a different cell at resting membrane potential. Horizontal lines are the means of these values; **, value significantly greater than for striatonigral neurons ($P < 0.01$).
neurons, it might be present on a proportionally higher number, i.e., as many as 45% of neurons in this efferent cell group. This estimate is within the range of other investigators who have examined the frequency of occurrence of D2 receptor markers on striatonigral neurons (6, 7).

There is a possibility that retrograde labeling might not distinguish the subset of medium spiny neurons projecting to the nigra that also give off axon collaterals in the globus pallidus (21, 22). Thus, some striatal neurons retrogradely labeled from the globus pallidus might also have projected to the substantia nigra and vice versa. This possibility is unlikely to affect our results for two reasons. When both striatal targets were injected with different fluorescent labels, we rarely observed double-labeled striatal neurons, and no such cells were included in our data. Moreover, if a small number of dualy projecting neurons had been included in our data, this would not change the fact that the channel was expressed on cells projecting to the substantia nigra.

Differences in the frequency of encountering the 85-pS K\(^+\) channel on the two efferent groups may also imply differences in the level of expression or functionality of D2 receptors. To evaluate this, we compared the mean open probabilities of single channels and found that channels on striatopallidal neurons had a significantly higher \(P_o\) than those on striatoni- gral cells. This may be indicative of differences in the gating of the channel by D2 receptors in the two cell groups. The higher \(P_o\) values, taken together with the greater frequency with which we encountered active channels on striatopallidal cells, are consistent with the idea that there is a higher degree of D2 receptor modulation of this channel in striatopallidal than striatonigral neurons. This might result from a higher receptor density, a more efficient mechanism of coupling between the receptor and the channel, different subtypes of D2-like receptors in the two cell groups, or a combination of the above. In previous studies in which we recorded from striatal neurons that were not retrogradely labeled (18), we observed this channel in about 25% of patches, a value intermediate between those found for the two cell groups in our present study and, thus, perhaps an average of the mixed cell types present in the dissociated cell preparation. We also had noted a heterogeneity of \(P_o\) values, which our present results indicate may have been a result of differences between the two cell groups. In other studies, we have found that the channel is activated by 7-OH-DPAT, a D\(_3\)/D\(_2\) dopamine receptor agonist, with a similar heterogeneity in \(P_o\) values (23). This raises the possibility that expression of D\(_3\) versus D\(_2\) receptors (15), or gating of the channel mediated by these receptor subtypes, may differ between the two efferent populations.

Other investigators have employed whole-cell recordings from similarly prepared neurons to assess the issue of D1 and D2 receptor coexpression. Our findings of D2-mediated electrophysiological responses on striatonigral neurons are consistent with, and extend, these previous studies. Specifically, Na\(^+\) currents of acutely dissociated, retrogradely labeled striato- nigral neurons were modulated frequently by both D1 and D2 receptor agonists (4, 24, 25). In other studies, more than half of acutely isolated striatal medium spiny neurons showed modulation of voltage-dependent Ca\(^{2+}\) currents by both classes of agonists (5, 24). However, we have been able to further evaluate differences between the two cell groups by performing our recordings at the single-channel level. On the one hand, we found that 85-pS K\(^+\) channels were very similar in conductance and reversal potential between the two cell groups, and so it is unlikely that the two kinds of cells express different forms of this D2-modulated K\(^+\) channel. On the other hand, the gating of the channel when modulated by D2 receptors, as determined from fractional open probabilities, was significantly different, suggesting differences in receptor-channel coupling efficiency. This result points toward a more subtle difference between the two cell groups than would result from complete receptor segregation. Because whole-cell recordings do not readily distinguish between channel conductance and gating, such information is best obtained from single-channel recordings. In addition, whole-cell recordings necessarily perfuse the interior of the cell with the patch-pipette solution. Since D1 and D2 receptors can exert opposing effects on second messenger systems (17), cell-attached recordings also have the advantage of not disturbing the intracellular milieu and therefore are not as likely to alter the relative contributions of the two receptors to the response.

Our studies did not directly measure the presence of D1-like receptors or D1-mediated effects on striatal neurons. Consequently, we are unable to estimate the extent to which striatopallidal neurons, a high percentage of which are known to express D2 receptors (2, 9), might also coexpress D1 receptors. However, our detection of a D2-mediated response on striatonigral neurons, a high percentage of which are known to express D1 receptors (2, 10, 26), makes it very likely that a significant number of striatoni- gral neurons are regulated exclusively by D1 receptors or that dopamine exerts opposite effects on the two cell populations. It thus is necessary to revise our understanding of basal ganglia information processing to allow for D1 and D2 receptor interactions on single striatal efferent neurons.

It is not clear how the discrepant estimates of the extent of D1/D2 coexpression by various investigators might be reconciled. It is possible that the balance of expression of the two receptors on the efferent populations may be influenced by the status of the dopamine system. Coexpression may be more common in normal animals with an intact dopamine system (3–8, 11), whereas a shift toward a more segregated expression of the receptors may occur as a result of dopamine-depleting (6-hydroxydopamine) lesions (2). If this were true, then interactions between coexpressed D1 and D2 receptors might be viewed as playing a Normal and important role in shaping the activity of both the “direct” striatonigral and “indirect” striatopallidal efferent pathways (27) and might contribute to the cooperative or synergistic interactions between the receptors that have been observed in behavioral and electrophysiological studies (28, 29).

In summary, we have shown that D2 receptors are not restricted exclusively to the striatopallidal pathway, but are likely to coexist with D1 receptors on a significant proportion of striatonigral neurons. Although these results are not consistent with a segregated view of D1 and D2 receptor expression on the two efferent populations, they remain in general agreement with a key feature of the basal ganglia model in that D2 receptors were found to be present on a higher proportion of, or in a higher density on, pallidal- versus nigral-projecting striatal neurons. D2 receptors therefore may be likely to play a greater role in regulating striatopallidal than striatonigral output. However, the likely coexpression of the two receptors on sizable subpopulations of striatal efferents makes it necessary to revise our views of basal ganglia efferent processing to include a role for D1/D2 interactions on single neurons.

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