D1/D5 Dopamine Receptors Stimulate Intracellular Calcium Release in Primary Cultures of Neocortical and Hippocampal Neurons

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Received 29 June 2001; accepted in final form 12 November 2001

Lezcano, Nelson and Clare Bergson. D1/D5 dopamine receptors stimulate intracellular calcium release in primary cultures of neocortical and hippocampal neurons. J Neurophysiol 87: 2167–2175, 2002; 10.1152/jn.00541.2001. D1/D5 dopamine receptors in basal ganglia, hippocampus, and cerebral cortex modulate motor, reward, and cognitive behavior. Previous work with recombinant proteins revealed that in cells primed with heterologous Gq/11-coupled G-protein-coupled receptor (GPCR) agonists, the typically Gs-linked D1/D5 receptors can stimulate robust release of calcium from internal stores when coexpressed with calyoxin. To learn more about the intracellular signaling mechanisms underlying these D1/D5 receptor regulated behaviors, we explored the possibility that endogenous receptors stimulate internal release of calcium in neurons. We have identified a population of neurons in primary cultures of hippocampus and neocortex that respond to D1/D5 dopamine receptor agonists with a marked increase of neurons in primary cultures of hippocampus and neocortex that respond to D1/D5 dopamine receptor agonists with a marked increase in intracellular calcium (Ca2+). The D1/D5 receptor stimulated responses occurred in the absence of extracellular Ca2+ indicating the rises in Ca2+ involve release from internal stores. In addition, the responses were blocked by D1/D5 receptor antagonists. Further, the D1/D5 agonist-evoked responses were state dependent, requiring priming with agonists of Gq/11-coupled glutamate, serotonin, muscarinic, and adrenergic receptors or with high external K+ solution. In contrast, D1/D5 receptor agonist-evoked Ca2+ responses were not detected in neurons derived from striatum. However, D1/D5 agonists elevated cAMP levels in striatal cultures as effectively as in neocortical and hippocampal cultures. Further, neither forskolin nor 8-Br-cAMP stimulation following priming was able to mimic the D1/D5 agonist-evoked Ca2+ response in neocortical neurons indicating that increased cAMP levels are not sufficient to stimulate Ca2+ release. Our data suggest that D1-like dopamine receptors likely modulate neocortical and hippocampal neuronal excitability and synaptic function via Ca2+ as well as cAMP-dependent signaling.

INTRODUCTION

Dopamine (DA) transmission is mediated by five G-protein-coupled receptors (GPCR) classified as either D1-like (including the D1 and D5 subtypes) or D2-like (including the D2–D4 subtypes). The D1 subtype is the most abundant DA receptor in brain, and the importance of the D1-like receptors in modulating motor (Gerfen 2000), cognitive (Williams and Goldman-Rakic 1995; Zahrt et al. 1997), and reward (Self et al. 1996; Smith-Roe and Kelley 2000) behavior is well established. At the cellular level, D1/D5 DA receptor agonists regulate neuronal excitability by altering ion channel activity. In addition, there is evidence that D1-like receptors can modulate various forms of synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), in neocortex (Gurden et al. 2000; Otani et al. 1998), hippocampus (Huang and Kandel 1995; Matthies et al. 1997; Otmakova and Lisman 1998), and striatum (Calabresi et al. 1992).

Agonist stimulation of recombinant D1 and D5 DA receptors in heterologous systems results in cyclic 3’-5’ AMP (cAMP) accumulation due to coupling with the heterotrimeric G protein α subunit, Gs (Grandy et al. 1991; Zhou et al. 1990). Additionally, in neurons derived from dorsal striatum, D1/D5 receptor agonists regulate Ca2+ channel activity via a signaling cascade including cAMP-dependent kinase (PKA), DARPP-32, and protein phosphatase I (Surmeier et al. 1995). However, most examples of D1/D5 DA receptor mediated neuromodulation are not exclusively cAMP or PKA dependent. For example, L-type calcium channel inhibitors block D1/D5 receptor enhancement of NMDA receptor currents in dorsal striatum (Cepeda et al. 1998; Hernandez-Lopez 1997). In contrast, D1/D5 receptor potentiation of NMDA currents in nucleus accumbens (ventral striatum) is blocked by inhibitors of protein kinase C (PKC) (Chergui and Lacey 1999) but not inhibitors of PKA (Harvey and Lacey 1997). The mechanism(s) by which D1/D5 receptors modulate sodium currents in pyramidal neurons in frontal cortex is more controversial. Whereas Gorelova and Yang (2000) report that D1/D5 agonists increase persistent sodium currents in slices of rat prefrontal cortex via a mechanism involving PKC, Maurice et al. (2001) show that D1/D5 receptors do not alter persistent currents in acutely dissociated cortical neurons but rather inhibit rapidly inactivating sodium currents via a PKA-dependent mechanism. In contrast, D1/D5 receptor modulation of sodium currents of acutely dissociated hippocampal neurons displays both PKA- and PKC-dependent components (Cantrell et al. 1997, 1999a).

The ability of D1/D5 receptor agonists to modulate ion channel activity is also contingent on the physiological state of the neuron under consideration. For example, the effects of D1/D5 agonists on sodium channels in hippocampal (Cantrell et al. 1997, 1999) and neocortical neurons (Gorelova and Yang 2000; Maurice et al. 2001) are detectable at depolarized membrane potentials but not at normal resting membrane potentials. Nor are these effects detected when cells are hyperpolarized. Similarly, D1/D5 receptor agonists potentiate NMDA receptor currents (Cepeda et al. 1998; Hernandez-Lopez et al. 1997) and
Possible explanations for the mechanistic heterogeneity in the D1/D5 DA receptor-evoked responses include brain regional or cellular differences in receptor subtype affinity for DA (Tiberi and Caron 1994), subcellular distribution (Bergson et al. 1995; Ciliax et al. 2000; Yung et al. 1995), and G protein coupling (Zhuang et al. 2000). Alternatively, interactions with activity modifying accessory proteins could alter endogenous D1 and/or D5 receptor function, potentially accounting for the diverse signaling mechanisms observed in native systems. For example, agonist-dependent interaction between D5 DA receptors and GABA<sub>A</sub> channels in heterologous cells was found to potentiate both cAMP production and chloride currents (Liu et al. 2000). Thus it seems possible that such an interaction underlies D1/D5 receptor agonist enhancement of chloride currents in striatal cholinergic neurons (Yan and Surmeier 1997), but this idea remains to be directly tested. Enhanced D1/D5 receptor signaling is also observed in heterologous cells as the result of interaction with calcyon, a single transmembrane protein which enables the receptors to stimulate intracellular calcium (Ca<sup>2+</sup>) release (Lezcano et al. 2000).

Here we explore the possibility that endogenous D1/D5 receptors stimulate Ca<sup>2+</sup> release by fura-2 ratiometric imaging of neurons in primary cultures of neonatal rat. We find that D1/D5 DA receptor agonists elicit Ca<sup>2+</sup> release in neurons derived from neocortex and hippocampus, but not striatum. The D1/D5 receptor stimulated Ca<sup>2+</sup> response required priming of cells with agonists of G<sub>q/11</sub> coupled adrenergic, glutamatergic, serotonergic, or muscarinic receptors, or with high external K<sup>+</sup> solutions. The D1/D5 receptor Ca<sup>2+</sup> response in neurons cannot fully be explained by increased levels of cAMP as D1/D5 agonists stimulated cAMP production in neocortical and striatal neurons to a similar extent. Taken together, our results indicate that Ca<sup>2+</sup> signaling plays a role in D1-like DA receptor modulation of neuronal excitability and synaptic processes in neocortex and hippocampus.

**METHODS**

**Primary cell cultures**

Neurons were isolated and cultured as described (Bekkers and Stevens 1991) with some modifications. Briefly, the frontal cortex, hippocampus or striatum (Paxinos and Watson 1998) was dissected from newborn rats and immediately immersed in ice-cold dissecting medium (Earle’s balanced salt solution containing 10 mM HEPES and 1 mM pyruvate, pH 7.3) for removal of blood vessels, meninges, and white matter. The tissue was minced, transferred to MEM containing papain (25 U/ml; Worthington), and incubated at 37°C in a 5% CO<sub>2</sub> incubator for 1 h with gentle rocking. Papain was removed by washing twice with inactivation solution [MEM containing 10% fetal bovine serum (FBS)], and the tissue dissociated by trituration. After centrifugation, cells were resuspended in growth medium [MEM containing 2% B-27, 0.1% mito serum extender, 5% FBS, 0.6% glucose, 1 mM pyruvate, 1 mM glutamine, penicillin (50 U/ml) and streptomycin (50 μg/ml)], counted and plated at a density of 25,000 cells/ml on glass coverslips treated with high molecular weight poly-l-lysine (Sigma Chemical) for Ca<sup>2+</sup> imaging, or on 35-mm tissue culture dishes for cAMP assays.

**Ca<sup>2+</sup> imaging**

Cell cultures (4–10 days in vitro) were rinsed with HBS (150 mM NaCl, 10 mM NaHEPES, 10 mM glucose, 2.5 mM KCl, 4 mM CaCl<sub>2</sub>, and 2 mM MgCl<sub>2</sub>, pH 7.4), then loaded with 5 μM Fura-2 AM (Gryniewicz et al. 1985) (Molecular Probes) in HBS at RT. After 20 min, cells were washed three times with HBS. Assays were performed at RT in 1.5 ml of HBS. Drugs were prepared in HBS and manually applied; and a perfusion apparatus was engaged to change solutions in the chamber. Samples were analyzed with a Zeiss Axiosvert 135 microscope 1 (×40 objective). Images were collected via a CCD camera (PXL, Photometrics) connected to a Silicon Graphics workstation using 4 × 4 binning. Samples were sequentially illuminated with a 75-W Zeiss XBO xenon lamp at 5-s intervals for 50–60 ms, first at 340 nm and then at 380 nm. Fluorescence emission at 510 nm was monitored for each excitation wavelength, and analyzed with Inovision-Ratiotool 4.3.5 software. Pixel intensities within selected areas of the images (with each area corresponding to a single neuron) were digitized for both wavelengths at each time point of the experiment.

**cAMP measurement**

Neocortical and striatal cultures (4–10 days in vitro) were washed twice with HBS and then exposed to agonists, SKF-81297-HBr (10 μM; Sigma), S-3,5-dihydroxyphenyl-glycine (DHPG) (50 μM; Tocris), carbachol (10 μM; Sigma), or forskolin (5 and 20 μM; Sigma) at room temperature in HBS. Phosphodiesterases were blocked by addition of Ro 20–1724 (100 μM; Sigma). After timed incubation, cells were placed on ice, washed once in cold PBS, then lysed by addition of HCl. cAMP levels in the supernatants were determined by direct cAMP enzyme immunoassay kit (Assay Designs). Pellets were resuspended in boiling 10% SDS; protein concentrations were determined following dilution of SDS to 0.9% by addition of 10 mM Tris pH 7.4 using BCA protein assay reagents (Pierce).

**RESULTS**

D1/D5 DA receptor agonists were bath applied to primary cultures of rat frontal cortex, hippocampus and striatum loaded with Fura-2 AM (Gryniewicz et al. 1985) to test whether the endogenous D1-like DA receptors stimulate Ca<sup>2+</sup> release. However, neither the D1/D5 receptor agonist SKF81297 (10 μM) nor SKF38393 (500 μM) evoked a detectable rise in F<sub>340</sub>/F<sub>380</sub> nm in any of the 25 neurons tested in each type of culture. Previous studies indicated that recombinant D1 or D5 DA receptors could stimulate Ca<sup>2+</sup> release when heterologously expressed if the transfected cells were previously stimulated (primed) with endogenous G<sub>q/11</sub>-coupled GPCR agonists (Lezcano et al. 2000). Therefore we next asked if D1/D5 receptor agonists could stimulate a response in neuronal cultures following activation of G<sub>q/11</sub>-coupledGPCRs.

**Native D1/D5 DA receptors evoke Ca<sup>2+</sup> transients in primed hippocampal and neocortical but not striatal neurons**

The G<sub>q/11</sub>-linked group I glutamate receptor subtypes, metabotropic glutamate receptor 1 (mGluR1) and mGluR5, have been localized in dendritic spines and shafts (Lujan et al. 1996; Romano et al. 1995), similar to the D1-like DA receptors (Bergson et al. 1995; Smiley et al. 1994; Yung et al. 1995). Therefore we tested whether the group I mGluR agonist, DHPG might prime a D1/D5 receptor-stimulated Ca<sup>2+</sup> response. Bath application of DHPG (50 μM) evoked an immediate rise in Ca<sup>2+</sup> in some of the neocortical, hippocampal and...
striatal neurons imaged (Fig. 1). When applied following return of Ca$^{2+}$ toward baseline levels, both D1/D5 receptor agonists, SKF38393 (500 μM) and SKF81297 (10 μM), were also able to stimulate an immediate rise in Ca$^{2+}$ in some of the DHPG-responsive neurons. D1/D5 DA receptor-stimulated Ca$^{2+}$ responses were detected in neocortical and hippocampal neurons (Fig. 1, A and B). In contrast, neither the D1 agonist SKF81297, nor SKF38393 was able to stimulate a rise in Ca$^{2+}$ in any of the 50 DHPG-responsive striatal neurons (Fig. 1C).

Similar to the DHPG-stimulated Ca$^{2+}$ responses, the SKF81297- and SKF38393-evoked responses in hippocampal and neocortical neurons peaked within 5–10 s of agonist application (Fig. 1, A and B). In addition, as with DHPG, the D1/D5 agonists stimulated increases in Ca$^{2+}$ levels within the cell body and neuronal processes radiating from the soma (Fig. 2, A, D, and E). However, due to the time resolution of our measurements, we cannot rule out the possibility that the increases in F$_{340}$/F$_{380}$ detected in processes resulted from diffusion of Ca$^{2+}$ and/or Ca$^{2+}$-bound dye from the cell body.

After identifying DHPG and D1/D5 agonist responsive neurons, neocortical and hippocampal cultures were perfused with Ca$^{2+}$-free media to determine whether extracellular and/or intracellular Ca$^{2+}$ contributed to the rise in Ca$^{2+}$ levels evoked by the D1/D5 receptor agonists. As shown in Fig. 2B, DHPG evoked reduced, and in some instances undetectable, responses in Ca$^{2+}$-free media compared with the responses evoked in media containing Ca$^{2+}$ possibly because group I mGluRs are capable of regulating membrane Ca$^{2+}$ channels (Fagni et al. 2000). In contrast, subsequent reapplication of D1/D5 agonists evoked responses in Ca$^{2+}$-free media similar in magnitude to those obtained in media containing Ca$^{2+}$, suggesting that D1/D5 agonists stimulate release of Ca$^{2+}$ from vesicular stores (Fig. 2B). Further, the Ca$^{2+}$ fluxes depended on functional D1/D5 DA receptors because addition of a D1-like receptor antagonist SCH23390 (5 μM) after perfusion with buffer, and prior to reapplication of DHPG, blocked the ability of the neurons to respond again to SKF81297 or SKF38393 (Fig. 2C).

Carbachol application also produced rises in Ca$^{2+}$ in neocortical, hippocampal, and striatal neurons presumably via activation of G$q/11$-coupled m1, m3, and m5 muscarinic receptors (Levey et al. 1993; Mrzljak et al. 1993; Wall et al. 1991; Yasuda et al. 1993). D1/D5 receptor agonists stimulated detectable rises in Ca$^{2+}$ in hippocampal or neocortical neurons primed with carbachol (10 μM; Fig. 3, A and B), but not in striatal neurons responding to carbachol (Fig. 3C). To confirm that priming was necessary for the D1/D5 agonist-stimulated responses, responsive neurons were perfused with buffer to remove drugs, and SKF81297 reapplied. Whereas a response to SKF81297 was detectable if the D1/D5 agonist was reapplied after priming with carbachol, none was detected following reapplication of the D1/D5 agonist alone (Fig. 3A).

We also asked whether a wider spectrum of neurotransmitters signaling through G$q/11$-coupled GPCRs might set the stage for the D1/D5 agonist-stimulated rises in neuronal Ca$^{2+}$ levels. Expression of G$q/11$-coupled 5HT$_{2A}$ and 5HT$_{2C}$ serotonergic (Cornea-Hebert et al. 1999; Vysokanov et al. 1998) and α$_3$ adrenergic (Gioanni et al. 1998) receptors has been detected in neocortical neurons. Therefore neocortical neurons were stimulated with D1/D5 receptor agonists following application of either the 5HT$_2$ receptor agonist, α-methyl 5HT (100 μM), or the α$_{1A/D}$ adrenergic receptor agonist, methoxamine (100 μM). As shown in Fig. 3, D and E, both α-methyl 5HT and methoxamine stimulated an increase in F$_{340}$/F$_{380}$, and effectively primed a similar response to the D1/D5 receptor agonist, SKF81297. In contrast, although the G$i/o$-linked β-adrenergic (Aoki et al. 1998) and G$q/11$-linked D2 DA (Gaspar et al. 1995) receptors have been localized in neocortical neurons, D1/D5 receptor agonists were unable to evoke Ca$^{2+}$ transients in any of 47 neocortical neurons primed by prior application of isoproterenol (10 μM) or quinpirole (1 μM), β-adrenergic and D2 DA receptor agonists, respectively (data not shown). Taken together, these studies suggest that a signaling step activated by G$q/11$-linked receptors is necessary for priming the ability of D1/D5 DA receptors to stimulate Ca$^{2+}$ release in neocortical and hippocampal neurons. Priming with G$q/11$-linked GPCR agonists, however, was not sufficient to enable D1/D5 receptors to stimulate Ca$^{2+}$ release as only approximately 44% of the DHPG-responding hippocampal (35/80) and cortical (68/154) neurons responded to subsequent D1/D5 receptor agonist application (Fig. 3F and prior figures). Likewise, SKF81297-stimulated increases in Ca$^{2+}$ were detected in approximately
30% of the carbachol-responding neurons (52/141 neocortical and 10/40 hippocampal neurons). The changes in fluorescence of the DHPG or carbachol responses \((F_{340}/F_{380})_{\text{peak}}/(F_{340}/F_{380})_{0}\) in neurons that did not also respond to D1/D5 receptor agonists varied widely but were not significantly different from the size of the responses to these agents in neurons which also responded to D1/D5 receptor agonists. Further, while the D1/D5 receptor Ca\(^{2+}\)/EGTA responses tended to be smaller than the DHPG or carbachol responses, the differences were not significant. Nevertheless, these results confirm that, in hippocampal and neocortical neurons, Ca\(^{2+}\) release, as a consequence of D1/D5 receptor stimulation, is a valid possibility.

**Inhibition of protein kinase C reduces D1/D5 DA receptor agonist stimulated Ca\(^{2+}\) release**

G\(_{q/11}\)-coupled GPCR stimulation leads to protein kinase C (PKC) activation via diacylglycerol (DAG) and Ca\(^{2+}\) generation (Oancea and Meyer 1998). The D1/D5 agonist stimulated Ca\(^{2+}\) response can typically be detected multiple times within the same neuron if preceded by application of a G\(_{q/11}\) GPCR agonist to prime the response (e.g., Fig. 3C). To test the possibility that activation of PKC may be involved in priming, a PKC inhibitor, bisindolylmaleimide I (2 \(\mu\)M), was applied to SKF81297-responsive neurons following perfusion with buffer and prior to reapplication of DHPG. The PKC inhibitor blocked the ability of the neurons to respond further to the D1/D5 receptor agonist but had no apparent effect on the DHPG response (Fig. 4A).

**D1/D5 DA receptor agonists stimulate Ca\(^{2+}\) release in high external K\(^+\) solutions**

To test whether changes in neuronal excitability resulting from ion channel activity can substitute for heterologous G\(_{q/11}\)-coupled GPCR-dependent priming, a high concentration K\(^+\) solution (100 mM final) containing tetrodotoxin (0.5 \(\mu\)M final) was applied to the bath to depolarize membranes. Intracellular Ca\(^{2+}\) levels rose immediately when the neurons were placed in high external K\(^+\). When the D1/D5 receptor agonist, SKF81297, was applied following return of Ca\(^{2+}\) levels to baseline, as shown in Fig. 4B, an immediate rise in Ca\(^{2+}\) levels was detected in some (approximately 10%) of the neocortical neurons depolarized by KC1.
Agents that increase cAMP do not mimic D1/D5 agonist stimulated Ca\(^{2+}\) release

Forskolin (20 µM) activates Ca\(^{2+}\) transients in striatal cultures prepared from 16- to 17-day-old rat embryos (Zanassi et al. 2001). It seemed possible therefore that cAMP may play a role in the ability of D1/D5 DA receptor agonists to evoke a Ca\(^{2+}\) response in neocortical and hippocampal but not striatal neurons isolated and cultured from neonatal rat. Thus we measured cAMP levels in the neocortical and striatal cultures following treatment with the D1/D5 receptor agonist, SKF81297 (10 µM) alone, or after priming with DHPG (50 µM) or carbachol (not shown; Fig. 4). Both types of D1/D5 receptor agonist application resulted in a 50–100% increase in cAMP levels in both cultures, whereas forskolin (5 or 20 µM) treatment increased cAMP levels in both neocortical and striatal cultures by approximately 150%. However, the differences in cAMP accumulation in the striatal versus the neocortical cultures were not significant for any of the treatments (P > 0.05). To further test whether elevating cAMP is sufficient for the D1/D5 receptor-evoked Ca\(^{2+}\) response at the single-cell level, forskolin (5 and 20 µM) or 8-Br-cAMP (100 µM) was applied to D1/D5 agonist-responsive neocortical neurons following washing and DHPG stimulation. However, neither forskolin nor 8-Br-cAMP (not shown) elicited a detectable rise in F\(_{340}/F_{380}\) in any of the 25 D1/D5 agonist-responsive neocortical neurons (Fig. 4D). Likewise, neither forskolin nor 8-Br-cAMP, applied after DHPG or carbachol, was able to stimulate a Ca\(^{2+}\) response in neurons within the striatal cultures (data not shown). In addition, we also tested whether an additional cAMP-dependent mechanism of priming the D1/D5 receptor Ca\(^{2+}\) response may obtain in striatal or neocortical cultures by applying forskolin prior to application of SKF81297. However, application of D1/D5 receptor agonists after forskolin (20 µM) did not elicit a Ca\(^{2+}\) response in any of the 26 neocortical or 38 striatal neurons tested although forskolin, itself, stimulated an increase in F\(_{340}/F_{380}\) in two of the neocortical and in six of the striatal neurons.

**DISCUSSION**

We report here that the D1/D5 DA receptor-selective agonists, SKF81297 and SKF38393, can activate intracellular Ca\(^{2+}\) release in cell bodies of neocortical and hippocampal neurons in primary culture, a response that can be blocked by SCH23390, a D1/D5 DA receptor antagonist. Heterologously expressed D1/D5 DA receptors typically stimulate G\(_{i}\) (Grandy et al. 1991; Zhou et al. 1990) resulting in cAMP accumulation. Yet the pharmacology of the responses we detect clearly indicates that endogenous D1-like DA receptor signaling also results in increased Ca\(^{2+}\) levels. Either, or both, of the known D1-like subtypes may be involved in the observed Ca\(^{2+}\) re-
responses because both D1 and D5 receptors are expressed in neocortex and hippocampus (Bergson et al. 1995; Ciliax et al. 2000; Montague et al. 2001). It is also possible that the Ca\(_{2+}\) responses are stimulated by an as yet unidentified D1/D5 agonist responsive receptor subtype.

The D1/D5 agonist-stimulated rises in Ca\(_{2+}\) were independent of extracellular Ca\(_{2+}\), suggesting that the underlying mechanism involves release from vesicular stores. The conventional mechanism by which GPCRs stimulate Ca\(_{2+}\) release involves stimulation of G\(_{q/11}\) and resultant phospholipase C\(\beta\) (PLC\(\beta\)) catalyzed hydrolysis of phosphoinositols 4,5-bisphosphate (PIP\(_2\)) to inositol 1,4,5-triphosphate (IP\(_3\)) (Hamm 1998). Binding of IP\(_3\) to IP\(_3\) receptors localized on vesicular stores opens these channels, resulting in increased cytosolic Ca\(_{2+}\) levels (Berridge et al. 1998). Although physical association of D1/D5 agonist binding sites and G\(_{q/11}\) has been reported (Wang et al. 1995), it is unclear whether native D1/D5 DA receptors stimulate Ca\(_{2+}\) release via PLC\(\beta\). Indeed, data from heterologous expression studies are consistent with D1 receptors stimulating PIP\(_2\) hydrolysis and/or Ca\(_{2+}\) release by other mechanisms. For example, in LTK\(^{-}\) cells, which lack PLC\(\beta\), it was proposed that transfected D1 receptors stimulate PIP\(_2\) hydrolysis via PLC\(\gamma\) as part of a CAMP-dependent mechanism (Yu et al. 1996). In another study involving transfected HEK293 cells, it was reported that D1 receptors can stimulate Ca\(_{2+}\) release in a CAMP-dependent but PIP\(_2\) hydrolysis-independent manner (Lin et al. 1995). However, we find that both forskolin and 8-Br-cAMP are unable to mimic D1/D5 agonist-evoked responses, suggesting that CAMP formation is not sufficient for native D1/D5 DA receptor-stimulated Ca\(_{2+}\) release. D1/D5 DA receptor agonist-stimulated increases in inositol phosphates have previously reported in brain homogenates (Undie and Friedman 1990; Undie et al. 1994). In those studies, significant increases in IP\(_3\) levels were detected 0.5–4 h after SKF38393 application. In contrast, carbachol stimulated increases in IP\(_3\) levels peaked within 4 min of application presumably due to activation of G\(_{q/11}\)-linked muscarinic receptors in the brain homogenates (Undie and Friedman 1990). Although the SKF81297- and SKF38393-stimulated calcium responses follow a time course similar to that of the increases in IP\(_3\) reported for carbachol, future studies are necessary to analyze IP\(_3\) levels in the D1/D5 agonist Ca\(_{2+}\)-responsive neurons.

The D1/D5 receptor Ca\(_{2+}\) response detected in neocortical and hippocampal neurons is state dependent, requiring priming. Priming agents included agonists of putative G\(_{q/11}\)-coupled adrenergic, glutamatergic, serotonergic, or muscarinic receptors as well as high extracellular K\(^+\). Thus the ability to independently elevate Ca\(_{2+}\) levels appears to be a common feature of effective priming. However, the opening of voltage-dependent Ca\(_{2+}\) channels in the presence of high extracellular K\(^+\) would also be expected to result in release neurotransmitter containing synaptic vesicles (Fossier et al. 1999). Release of neurotransmitter could activate G\(_{q/11}\)-coupled GPCRs and potentially prime the D1/D5 Ca\(_{2+}\) response via a mechanism similar to that of carbachol, DHPG, \(\alpha\)-methyl 5HT, or methoxamine. That the D1/D5 receptor Ca\(_{2+}\) response was detected less frequently in neurons primed with high external K\(^+\) compared with G\(_{q/11}\)-coupled GPCR agonists suggests that priming may involve factors other than a transient increase in Ca\(_{2+}\) levels. G\(_{q/11}\) GPCR stimulation also results release of “free” B by and \(\alpha\) G protein subunits as well as formation of IP\(_3\) and
diacylglycerol (DAG) during PIP2 hydrolysis. As such, each of these G proteins, second messengers, or combination thereof, as well as Ca\(^{2+}\) and/or phospholipid-dependent isoforms of PKC, calcineurin (protein phosphatase 2B), or Cam kinase II (CamKII) could potentially play a role in priming D1/D5 DA receptor stimulated Ca\(^{2+}\) release. We find that bisindolylmaleimide I, a cell-permeant inhibitor of both the Ca\(^{2+}\) and/or DAG-dependent isoforms of PKC, blocks the ability of neurons to respond to D1/D5 receptor stimulation without altering the response to priming agents. These results suggest that PKC activity is necessary for effective priming, but does not rule out the possibility that PKC is required for the D1/D5 receptor Ca\(^{2+}\) response itself.

SKF81297 stimulated increases in cAMP levels indicated functional expression of D1-like receptors in the striatal cultures. However, in contrast to neocortical and hippocampal neurons, D1/D5 DA receptor agonists were unable to stimulate Ca\(^{2+}\) transients in striatal neurons although the neurons responded to group I mGluR agonists and carbachol. D1 and D5 receptors are primarily expressed in pyramidal neurons in cortex but, in GABAergic or cholinergic neurons in striatum (Bergson et al. 1995; Ciliax et al. 2000). Thus our results are consistent with the possibility of regional and/or cellular differences in the native D1/D5 receptor signaling in brain.

While the molecules regulating the D1/D5 receptor Ca\(^{2+}\) response in neocortex and hippocampus are not well defined, it is possible that regional differences in G protein coupling may underlie the differential signaling. For example, studies with G\(_{olf}\) knockout mice indicate that D1/D5 agonist responsive receptors likely couple to G\(_{olf}\) in striatum but to G\(_{i}\) in other brain regions (Zhuang et al. 2000). Further, D1/D5 DA receptors in cortex are less sensitive to guanine nucleotide regulation of agonist binding than those in striatum based on radioligand binding studies of human postmortem brain tissue (de Keyser et al. 1988). It is tempting to speculate regional differences in the expression of kinases or phosphatases involved in priming, or signaling molecules involved in the D1/D5 receptor Ca\(^{2+}\) response itself.

Several aspects of the native D1/D5 receptor-stimulated Ca\(^{2+}\) transients in neocortical and hippocampal neurons resembled the response evoked in HEK293 cells expressing calyceum and either of the recombinant D1-like DA receptor subtypes (Lezcano et al. 2000). Two key similarities are that the D1/D5 and either of the recombinant D1-like DA receptor subtypes bleb the response evoked in HEK293 cells expressing calcyon the expression of kinases or phosphatases involved in priming, et al. 1988). It is tempting to speculate regional differences in binding studies of human postmortem brain tissue (de Keyser et al. 1988). While the molecules regulating the D1/D5 receptor Ca\(^{2+}\) response in neocortex and hippocampus are not well defined, it is possible that regional differences in G protein coupling may underlie the differential signaling. For example, studies with G\(_{olf}\) knockout mice indicate that D1/D5 agonist responsive receptors likely couple to G\(_{olf}\) in striatum but to G\(_{i}\) in other brain regions (Zhuang et al. 2000). Further, D1/D5 DA receptors in cortex are less sensitive to guanine nucleotide regulation of agonist binding than those in striatum based on radioligand binding studies of human postmortem brain tissue (de Keyser et al. 1988). It is tempting to speculate regional differences in the expression of kinases or phosphatases involved in priming, or signaling molecules involved in the D1/D5 receptor Ca\(^{2+}\) response itself.

Several aspects of the native D1/D5 receptor-stimulated Ca\(^{2+}\) transients in neocortical and hippocampal neurons resembled the response evoked in HEK293 cells expressing calyceum and either of the recombinant D1-like DA receptor subtypes (Lezcano et al. 2000). Two key similarities are that the D1/D5 receptor evoked-response was not detectable in cells without prior activation of heterologous G\(_{q11}\)-coupled GPCRs (priming), and the response was cAMP independent. Further, D1/D5 receptor-stimulated Ca\(^{2+}\) release in calyceum transfected cells could be blocked by pretreatment with cell-permeant inhibitors of PKC. However, future studies will be necessary to determine whether interaction with calyceum is involved in D1/D5 DA receptor-stimulated Ca\(^{2+}\) release in neocortical and hippocampal neurons.

Release of Ca\(^{2+}\) from intracellular stores has been linked to LTP (Wilsch et al. 1998; Yeckel et al. 1999), LTD (Finch and Augustine 1998; Takechi et al. 1998), activity-dependent protein synthesis (Raymond et al. 2000; Weiler and Greenough 1993), and changes in dendritic spine shape (Korkotian and Segal 1999). Perhaps relevant to the physiological significance of the priming-dependent, D1/D5 receptor-stimulated Ca\(^{2+}\) response reported here, Otani et al. (1998) showed that LTD in neocortex could be produced by costimulation of metabotropic glutamate and dopamine receptors in the absence of high-frequency stimulation. Combined Ca\(^{2+}\) imaging and electrophysiological approaches should be able to determine whether endogenous D1-like DA receptors modulate synaptic processes in neocortex and hippocampus via mechanisms involving Ca\(^{2+}\) release.

We thank J. Jolly for critically reading the manuscript. This work was supported by Medical College of Georgia Combined Intramural Grants Program (N. Lezcano) and National Institute of Mental Health Grants MH-56608 and MH-63271 (C. Bergson).

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