0026-895X/09/7503-447–453
MOLECULAR PHARMACOLOGY
Vol. 75, No. 3
53017/3436733
Mol Pharmacol 75:447–453, 2009
Printed in U.S.A.

ACCELERATED COMMUNICATION

D_5 Dopamine Receptors are Required for Dopaminergic Activation of Phospholipase C

Asha Sahu, Kimberly R. Tyeryar, Habiba O. Vongtau, David R. Sibley, and Ashiwel S. Undieh

Laboratory of Integrative Neuropharmacology, Department of Pharmaceutical Sciences, Thomas Jefferson University School of Pharmacy, Philadelphia, Pennsylvania (A.S.U.); Molecular Neuropharmacology Section, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland (D.R.S.); and Department of Pharmaceutical Sciences, University of Maryland, Baltimore, Maryland (A.S., K.R.T., H.O.V.).

Received October 23, 2008; accepted December 1, 2008

ABSTRACT

Dopamine activates phospholipase C in discrete regions of the mammalian brain, and this action is believed to be mediated through a D_1-like receptor. Although multiple lines of evidence exclude a role for the D_1 subtype of D_1-like receptors in the phosphoinositide response, the D_5 subtype has not been similarly examined. Here, mice lacking D_5 dopamine receptors were tested for dopamine agonist-induced phosphoinositide signaling both in vitro and in vivo. The results show that hippocampal, cortical, and striatal tissues of D_5 receptor knockout mice significantly or completely lost the ability to produce inositol phosphate or diacylglycerol messengers after stimulation with dopamine or several selective D_1-like receptor agonists. Moreover, endogenous inositol-1,4,5-trisphosphate stimulation by the phospholipase C-selective D_1-like agonist 3-methyl-6-chloro-7,8-dihydroxy-1-[3methylphenyl]-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF83959) was robust in wild-type animals but undetectable in the D_5 receptor mutants. Hence, D_5 receptors are required for dopamine and selective D_1-like agonists to induce phospholipase C-mediated phosphoinositide signaling in the mammalian brain.

Multiple subtypes of dopamine receptors expressed in the mammalian brain may be categorized into D_1-like and D_2-like subclasses based on their structural homology, pharmacological selectivity, and functional similarities (Civelli et al., 1993; Lachowicz and Sibley, 1997). Members of the D_1-like subclass include the D_1 and D_5 receptors (Civelli et al., 1993; Lachowicz and Sibley, 1997). Expressed in clonal cell lines, both the D_1 and D_5 receptors have demonstrated coupling to multiple signaling cascades as assessed through the activation of specific G proteins or the formation of downstream second messengers (Sidhu and Niznik, 2000; Neve et al., 2004). However, promiscuous or inconsistent coupling to multiple or alternate G proteins and signaling cascades might be a frequent feature for many G protein-coupled receptors expressed in artificial cell lines (Sidhu and Niznik, 2000; Hermans, 2003). Thus, experiments aiming to definitively assign a signaling pathway to a given receptor would benefit from the use of physiological tissue preparations and receptor-selective pharmacological agents.

Although various chemical entities that are highly selective for D_1-like receptors exist, there remains a lack of agonists or antagonists that sufficiently discriminate between the D_1 and D_5 subtypes. Moreover, typical agonists of D_1-like receptors generally activate both the D_1 and D_5 subtypes and elicit multiple signaling responses in mammalian tissues (Neve et al., 2004). Such responses include cascades mediated by adenylyl cyclase (Monsma et al., 1990), mitogen-activated protein kinase (Zhen et al., 1998), intracellular calcium mobilization (Lezcano and Bergson, 2002; Yasumoto

This work was supported by the National Institutes of Health National Institute on Drug Abuse [Grant R01-DA017614] and by the Intramural Research Program of the National Institutes of Health.

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.

doi:10.1124/mol.108.053017.

ABBREVIATIONS: PLC, phospholipase C; PI, phosphatidylinositide; D_5KO, D_5 dopamine receptor knockout; HB, HEPES bicarbonate; CDP-diacylglycerol, cytidine diphosphate diacylglycerol; SKF38393, 1-phenyl-2,3,4,5-tetrahydro-(H1)-3-benzazepine-7,8-diol; SKF83959, 3-methyl-6-chloro-7,8-dihydroxy-1-[3methylphenyl]-2,3,4,5-tetrahydro-1H-3-benzazepine; ANOVA, analysis of variance; kb, kilobase.

et al., 2004), and phospholipase C (PLC) (Felder et al., 1989; Undie and Friedman, 1990). The PLC response, which is associated with the hydrolysis of phosphatidylinositides (PIs) to release inositol phosphate and diacylglycerol second messengers, is evident in discrete brain regions, including tissues expressing D_1-like receptors that show relatively negligible functional coupling to adenylyl cyclase (Undie and Friedman, 1990; Montague et al., 2001) and persists when D_1 receptors have been genetically deleted (Friedman et al., 1997) or chemically inactivated (Rosengarten and Friedhoff, 1998; Undie et al., 2000). Thus, although a D_1-like receptor mediates dopaminergic-induced PI signaling in the brain, the structurally defined D_1 receptor itself is not the specific subtype that is involved. Given that extensive data mining of mammalian genomes has not revealed the existence of additional dopamine receptors beyond the D_1–D_5 entities, the foregoing suggests that the D_5 subtype might be the D_1-like receptor that mediates the PLC response. This hypothesis has not been directly tested in the intact brain.

In the present work, we sought to further examine the nature of D_5 receptor coupling in native brain tissues. For this, we used mutant mice lacking D_5 receptors to test the hypothesis that the D_5 receptor is required for dopaminergic activation of PLC in the intact brain. The results demonstrate that dopamine and selective D_1-like receptor agonists lose their efficacy to induce inositol phosphate accumulation or diacylglycerol production in brain tissues of mice lacking the D_5 subtype of D_1-like receptors.

Materials and Methods

Animals. Dopamine D_5 receptor knockout (D_5KO) mice were obtained along with their wild-type litter mates from the National Institutes of Health (Bethesda, MD). Produced from a genetic background of 129/SvJ1 and C57BL/6J, the mice are viable, develop normally, and are fertile and capable of reproduction (Hollon et al., 2002). The genotype of each animal was determined by polymerase chain reaction techniques, and confirmed D_5KO animals showed complete loss of immunoreactivity for the D_5 receptor in the brain (Hollon et al., 2002). Mice used in this study were at least 10 weeks old. Protocols for the care and use of the animals were approved by the Institutional Animal Care and Use Committee and conformed to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Drugs and Chemicals. The D_1-like agonists SKF38393 and SKF83959 were obtained from the National Institute of Mental Health Chemical Synthesis Program (Bethesda, MD). Dopamine and the buffer reagents were purchased from Sigma-Aldrich (St. Louis, MO). Drugs were dissolved in saline and diluted into HEPES bicarbonate (HB) buffer (Undie and Friedman, 1990). Each experiment was performed on multiple occasions, each time using fresh preparations of drugs. Protein was assayed by the Bradford method using reagents from Bio-Rad Laboratories (Hercules, CA).

Assay of Phosphoinositide Hydrolysis in Brain Slices. Brain slices were prepared from wild-type and D_5KO mice as described previously (Undie and Friedman, 1992). Tissue slices were preincubated for 45 min in calcium-free HB buffer (Undie and Friedman, 1992), and then distributed in 25-μl aliquots (300 μg of protein) to 5-ml polypropylene tubes containing 150 μl of normal HB buffer (containing 1.2 mM $CaCl_2$). The assay tubes were aerated with 95% O_2/5% CO_2 and incubated at 37°C in a shaking water bath. Tissues were double-labeled with [^{3}H]inositol (6 μCi/ml) and [^{14}C]cytidine (0.6 μCi/ml) to, respectively, monitor the activities of inositol phosphates and the diacylglycerol metabolite CDP-diacylglycerol (Undie, 1999). After 30 min of the labeling reaction, 5 mM LiCl was added, followed 5 min later by the addition of indicated concentrations of dopamine or a D_1-like agonist. HB buffer was added as necessary to give a final incubation volume of 250 μl, and incubation continued for an additional 60 min. The reaction was stopped by mixing the slices with 1.5 ml of chloroform/methanol/1 M HCl (100:200:1). After incubating the mixture at room temperature for 45 min, followed by vigorous vortexing to complete the extraction process, the inositol phosphates and unreacted [^{3}H]inositol or [^{14}C]cytidine were partitioned from the phospholipids by sequential mixing with 0.5 ml of chloroform and 0.75 ml of deionized water. The mixture was vortexed for 3 min and then centrifuged at 1000*g* for 5 min. A 1-ml aliquot of the aqueous phase was used to assay the content of inositol phosphate species by Dowex anion exchange chromatography (Undie and Friedman, 1990). Chromatographic eluates were mixed with 8 ml of Scintisafe gel (Thermo Fisher Scientific, Waltham, MA) to convert the samples into gel form, and the tritium radioactivity was counted by liquid scintillation spectrometry. A 400-μl aliquot of the organic phase was quantitatively transferred into scintillation vials, allowed to dry overnight at room temperature, redissolved in 5 ml of Biosafe scintillation cocktail, and the tritium ([^{3}H]phosphoinositides) and C-14 ([^{14}C]CDP-diacylglycerol) activities were concurrently measured using the Beckman LS6500 spectrometer. Data for each analyte were converted to disintegrations per minute per microgram of protein as described previously (Undie and Friedman, 1990; Undie, 1999).

Assay of Endogenous Brain Phosphoinositide Hydrolysis. Agonist-induced release of endogenous inositol-1,4,5-trisphosphate was assayed ex vivo after in vivo drug treatments and subsequent excision of requisite test tissues. Mice were injected subcutaneously with 5 ml/kg saline (controls) or with selected dosages of test agents dissolved in 5 ml/kg saline. After allowing 15 min for the drug to act, the animals were rapidly decapitated, and the brains were removed and washed in ice-cold HB buffer. Brain regions of interest were dissected out, transferred into 2-ml Eppendorf tubes, and rapidly frozen in liquid nitrogen. If the tissues were not to be processed immediately, they were stored at −70°C until use. Frozen tissues were thawed to 4°C and homogenized in 1 ml of ice-cold 1 M trichloroacetic acid using a Polytron homogenizer (Kinematica, Littau-Lucerne, Switzerland) set at medium speed for 15 s. The tubes were placed on ice for 15 min and then centrifuged at 13,000*g* for 10 min at 4°C. The supernatant containing extracted inositol trisphosphate was transferred to another Eppendorf tube. The pellet was washed three times with distilled water, digested in 1 M NaOH, and the mixture was used for protein determination. To 0.5 ml of the supernatant was added 1 ml of a mixture of trichlorotrifluoroethane-trioctylamine (3:1), mixed vigorously for 15 s, and centrifuged for 1 min at 10,000*g*. An aliquot of the upper phase was used for inositol-1,4,5-trisphosphate determination by the radioreceptor binding assay using reagents and protocols from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK) (Cook et al., 1990). Inositol-1,4,5-trisphosphate content in the tissue samples is expressed in picomoles per milligram of tissue protein.

Data Analysis. Data for each brain region were normalized across experimental runs and then subjected to two-way analysis of variance (ANOVA) of second-messenger response by drug concentration by phenotype. Upon detecting statistical significance in the ANOVA results, post hoc Bonferroni analyses were performed to compare pairs of D_5KO and WT responses at each drug concentration (or in vivo dose). To determine whether any of the drug concentrations or doses exerted significant effects relative to baseline, post hoc Dunnett tests were performed. Statistical comparisons were considered significant at $p < 0.05$ or better.

Results

Effects of the D_5KO Phenotype on Dopamine Agonist Stimulation of Phosphoinositide Hydrolysis in Brain Slice Preparations. Using a range of drug concentrations

known to elicit dopaminergic stimulation of PI hydrolysis, we tested the effects of dopamine and selective D_1-like agonists in brain slice preparations of WT and D_5KO mice. The data are shown in Fig. 1. Two-way ANOVA analyses (inositol phosphate response by phenotype by drug concentration) for each drug indicated highly significant overall effects of concentration ($p < 0.001$) and phenotype ($p < 0.001$) on the second-messenger responses. Subsequent Bonferroni tests revealed that basal accumulation of inositol phosphate was not significantly different between wild-type and mutant frontal cortex or striatum but was significantly lower in the hippocampus ($p < 0.01$)—the brain region of greatest D_5 receptor expression (Montague et al., 2001).

Dopamine at the maximally effective concentrations of 100

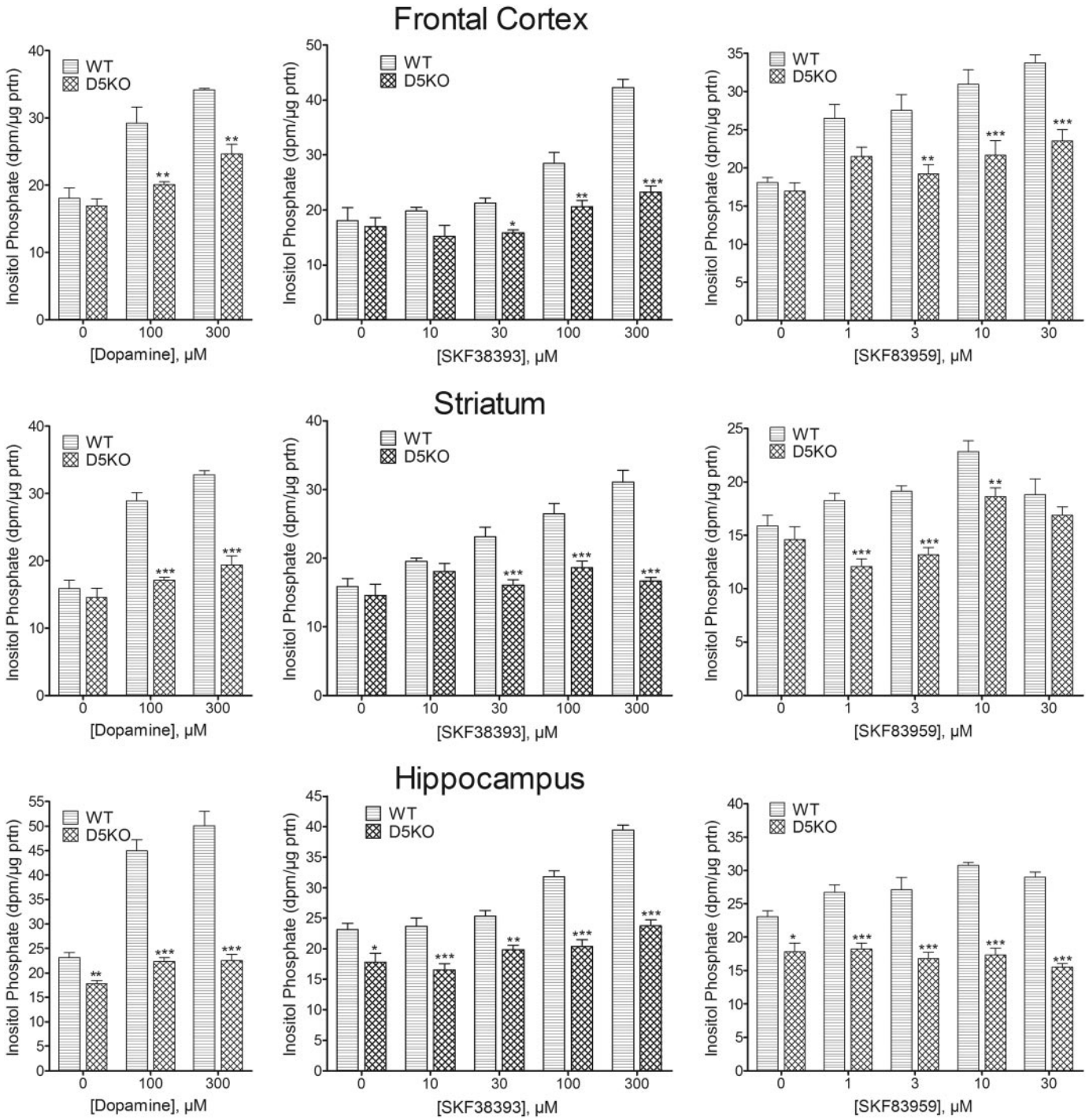

Fig. 1. Effects of D_5 receptor deletion on dopaminergic-induced inositol phosphate accumulation in brain slices. Tissue slices prepared from frontal cortex, striatum, or hippocampus of wild-type and D_5 receptor knockout (D_5KO) mice were double-labeled with [^{3}H]inositol and [^{14}C]cytidine and then incubated with indicated concentrations of test agonists for 60 min. Tritiated inositol phosphates were separated and quantified. Data from three to four separate experiments were normalized against baseline accumulations of [^{3}H]inositol phosphate in the corresponding WT tissues and then pooled for analysis. Each bar is the mean ± S.E.M. ($n = 6–8$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, post hoc Bonferroni comparisons of D_5KO versus WT responses at each drug concentration.

to 300 μM (Undie and Friedman, 1990) induced significant accumulations of inositol phosphate in brain slice preparations of wild-type mice ($p < 0.001$ for each tissue) and in the frontal cortex of mutant mice ($p < 0.01$). In each test tissue, however, drug effects in the mutant group were significantly reduced compared with the wild-type group ($p < 0.0001$; ANOVA main effects of phenotype for each drug and tissue type). Effects of the selective D_1-like receptor partial agonist, SKF38393, were significant and concentration-dependent, as would be expected, in the wild-type group; in the mutant group, however, the main ANOVA performed on only the mutant tissue data did not show any significant concentration-related effects of SKF38393 for any of the test tissues. With SKF83959, a selective D_1-like agonist that does not stimulate adenylyl cyclase but potently stimulates PI hydrolysis both in vitro and in vivo (Gnanalingham et al., 1995; Panchalingam and Undie, 2001; Jin et al., 2003), significant and concentration-related effects (up to 10 μM) were obtained in each tissue of wild-type animals ($p < 0.001$ for each tissue) but not in any of the tissues of the mutant group.

To test the diacylglycerol arm of the PI cycle, test tissue slices were concurrently labeled with [^{14}C]cytidine so that newly formed diacylglycerol may be converted to [^{14}C]CDP-diacylglycerol and thereby detected radiometrically (Undie, 1999). As shown in Fig. 2, tissues from wild-type mice responded to dopamine or the selective D_1-like agonists in a concentration-related manner, whereas the tissues from the mutant animals showed markedly reduced or absent effects. When data from the mutant tissues were isolated from the wild-type data and analyzed, there were no concentration-related effects for any of the drugs in any of the tissues except for the effects of dopamine in the mutant striatal tissues, in which the main ANOVA was significant ($p < 0.01$) and the 300 μM concentration differed significantly from control ($p < 0.01$). Again, there were no differences in basal accumulations of CDP-diacylglycerol among the phenotypes, with the exception of the hippocampus, in which loss of functional D_5 receptors was associated with a statistically significant decrease ($p < 0.05$) in basal levels of the product. Overall, the [^{14}C]CDP-diacylglycerol data (Fig. 2) were in agreement with the [^{3}H]inositol phosphate data (Fig. 1). Hence, the knockout phenotype markedly decreases or completely abolishes the ability of different D_1-like receptor-effective agonists to induce brain PI hydrolysis.

To test whether the effects of the D_5-knockout phenotype on PLC signaling were specific to the dopamine system, we examined the effects of the selective α-adrenergic receptor agonist, phenylephrine, on inositol phosphate and CDP-diacylglycerol accumulation in frontal cortex tissues. As shown in Fig. 3, phenylephrine significantly stimulated inositol phosphate and CDP-diacylglycerol in both the wild-type and the mutant tissues ($p < 0.001$ for each analyte in each phenotype). Bonferroni post hoc comparisons revealed no significant effect of phenotype on basal or phenylephrine-induced accumulation of inositol phosphate or diacylglycerol messengers.

Effects of SKF83959 on In Vivo Release of Inositol-1,4,5-Trisphosphate. In anticipation of a physiological relevance for the phenotypic differences in signaling efficacy, we tested the ability of a brain-accessible D_1-like agonist to induce second-messenger production in the brains of intact animals. Mice were administered behaviorally relevant dosages of the PLC-selective D_1-like agonist SKF83959 (Panchalingam and Undie, 2001; Jin et al., 2003). Endogenously formed inositol-1,4,5-trisphosphate levels were then measured in discrete brain tissues of wild-type and D_5 mutant mice, and the results are shown in Fig. 4. Basal levels of inositol-1,4,5-trisphosphate were between 65 and 85 pmol/mg protein in the test tissues; these values are within range of previous reports (Dwivedi et al., 2000; Jin et al., 2003). There were no significant differences in basal levels compared between wild-type and mutant animals in either the striatum or the hippocampus. SKF83959 induced significant and dose-dependent increases in inositol-1,4,5-trisphosphate levels in both the striatum and hippocampus of wild-type mice. The mutant animals, however, failed to show any significant increases in inositol-1,4,5-trisphosphate levels in response to the drug treatment. Apparently, the presence of D_5 receptors is required for SKF83959 to activate PLC-mediated inositol second-messenger formation in the intact brain.

Discussion

Dopaminergic stimulation of PI signaling has long been known to be mediated through a D_1-like receptor mechanism (Felder et al., 1989; Undie and Friedman, 1990). Related studies have excluded a role for the cloned D_1 receptor (Undie et al., 1994; Friedman et al., 1997), leaving the possibility that the D_5 receptor may be the subtype that mediates the PLC response. But this notion has never before been tested directly. The present findings show that the D_5 receptor is indeed crucial in the mediation of dopamine's stimulatory effects on PI metabolism. This inference is consistent with known characteristics of dopamine-induced PI signaling and D_5 receptor expression among the brain regions. For instance, as indicated in past and the present results, both receptor expression and agonist-induced signaling are relatively higher in the hippocampus than in the striatum, with intermediate effects in the prefrontal cortex (Undie and Friedman, 1990). Other consistent observations are those indicating that the D_5 receptor can couple to G_q-like G proteins in various cell lines or in renal brush-border membranes (Sidhu and Niznik, 2000), that the receptor directly modulates calcium currents and burst firing in the subthalamic nucleus (Baufreton et al., 2003), and it frequently exists in extrasynaptic microdomains associated with neuronal inositol-1,4,5-trisphosphate-sensitive calcium stores (Paspalas and Goldman-Rakic, 2004). The present findings, therefore, could provide new insights toward understanding those agonistic D_1-like effects that have hitherto defied explanation on the basis of adenylyl cyclase coupling.

Our current results are also supported by previous experiments involving the expression of a D_1-like receptor encoded by striatal mRNA in *Xenopus laevis* oocytes. Mahan et al. (1990) found that injection of rat striatal mRNA into *X. laevis* oocytes led to the expression of a D_1-like receptor coupled to inositol phosphate production and Ca^{2+} mobilization. However, expression of the cloned rat D_1 receptor in the oocytes lead to the production of cAMP but not Ca^{2+} mobilization, suggesting that the D_1 receptor was not linked to the PI response (Monsma et al., 1990). Moreover, using size fractionation techniques, it was shown that mRNA encoding the striatal PI-linked D_1-like receptor was between 2.5 and 3.0 kb in size, thus distinguishing it from the 4.1-kb mRNA fragment that encodes the rat D_1 receptor. It is interesting

that the rat D_5 receptor is encoded by an mRNA that is ~3 kb in size (Tiberi et al., 1991), in close agreement with the size of the mRNA encoding the PI-linked D_1-like receptor identified using the oocyte expression system. Our conclusion is that the Ca^{2+} response observed in these early oocyte expression experiments involved the D_5 receptor subtype.

Experiments with selective α-adrenergic receptor stimulation in frontal cortical tissues revealed that the effects of the D_5 receptor loss may be specific to the dopamine system rather than constitute a generalized deficit in response to PLC-coupled monoaminergic receptors. Moreover, there was general agreement between the in vitro and in vivo drug effects. Although baseline accumulations of inositol phosphate or CDP-diacylglycerol were lower in the mutant hippocampal slices compared with the wild-type tissue, no such differences were observed in the striatum or frontal cortex, and no hippocampal differences

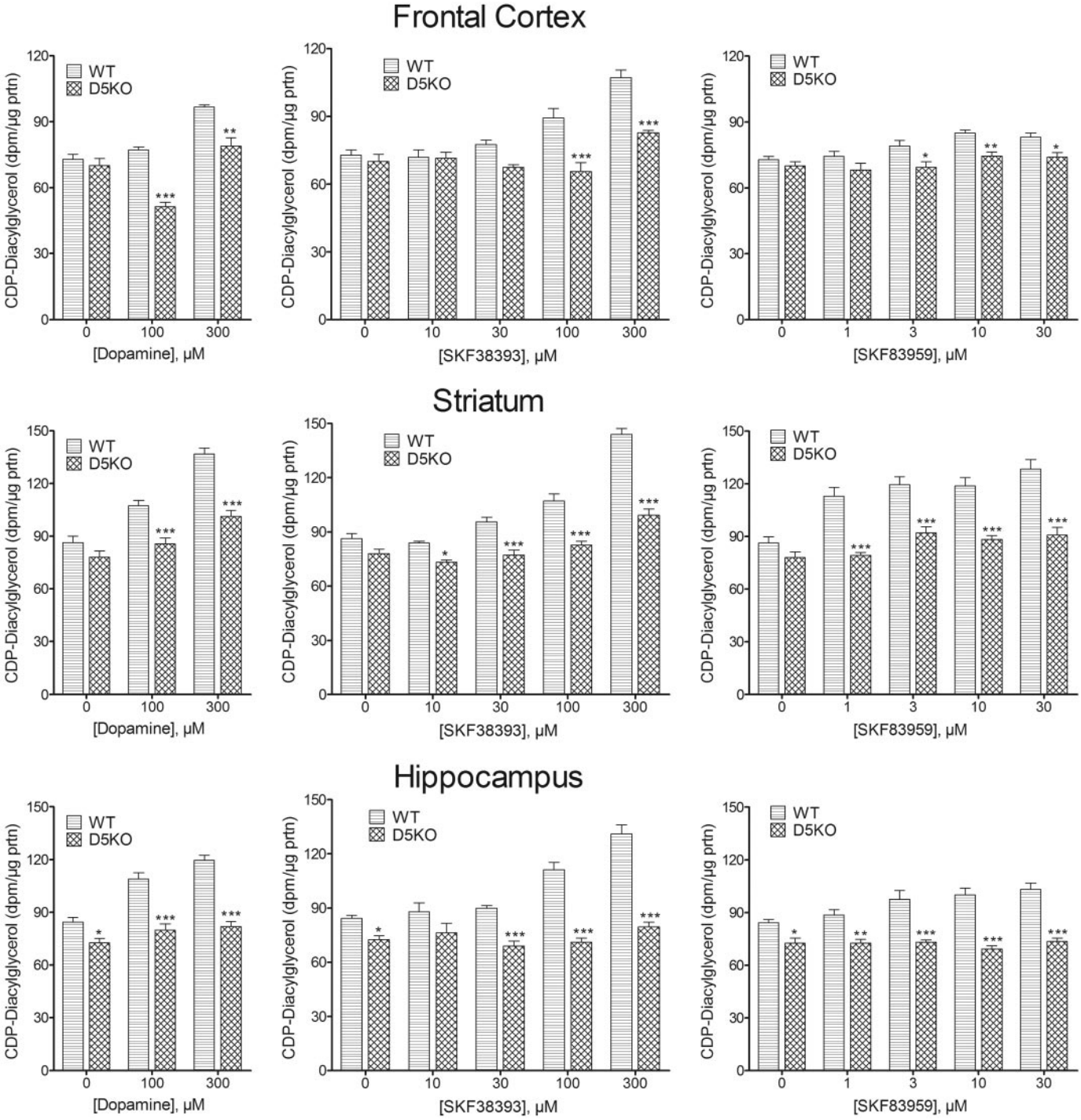

Fig. 2. Effects of D_5 receptor knockout phenotype on agonist-induced CDP-diacylglycerol stimulation in brain slices. Experiments were conducted as described in Fig. 1. Diacylglycerol-derived [^{3}H]CDP-diacylglycerol was extracted and quantified by liquid scintillation as described. Data for each tissue were collated from three to four separate experiments and normalized against wild-type baseline accumulations of [^{3}H]CDP-diacylglycerol. Each bar is the mean ± S.E.M. (n = 6–8). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, post hoc Bonferroni comparisons of D_5KO versus WT responses at each drug concentration.

were evident in the in vivo assay. Furthermore, factoring out the basal accumulation for each phenotype did not change the outcome of the analyses of drug effects, thus indicating that the difference in drug effects among the phenotypes outweighed any baseline differences.

For the in vivo studies, it was particularly noteworthy that phenotypic differences were observed with SKF83959, a selective D_1-like agonist that does not stimulate adenylyl cyclase but potently stimulates PI hydrolysis (Gnanalingham et al., 1995; Panchalingam and Undie, 2001; Jin et al., 2003). This suggests little if any role for cAMP activity in dopaminergic PLC stimulation.

Although pharmacological agents that sufficiently discriminate among the subtypes of D_1-like receptors are still lacking, there are, nevertheless, distinguishable anatomical, biochemical, and molecular characteristics within the D_1-like receptor subfamily. For instance, the D_5 receptor is widely but discretely distributed in the brain, with a pattern that differs substantially from the distribution of D_1 receptors (Meador-Woodruff et al., 1992; Ciliax et al., 2000). Within the striatum, the D_5 receptor is expressed predominantly in cholinergic interneurons, whereas D_1 receptors predominate in GABAergic neurons (Rivera et al., 2002; Centonze et al., 2003). Unlike the D_1 receptor, D_5 receptors do not form functional complexes with A_1 adenosine receptors (Le Crom et al., 2002), and plasma membrane localization of the D_5 (but not the D_1) receptor requires *N*-glycosylation (Karpa et al., 1999). These observations indicate significant structural differences between the receptor subtypes, and this is consistent with the recent observation of differential physicochemical susceptibility of G_s-coupled versus G_q-coupled D_1-like sites to reducing agents or plasma membrane perturbations (Panchalingam and Undie, 2005). Thus, although there may be substantial pharmacological overlap within the D_1-like family, the two subtype members are sufficiently differentiated in structure, anatomical distribution, and function to warrant their differential coupling to downstream signaling cascades. Nevertheless, future studies on the D_5 receptor should address whether such coupling ultimately involves receptor hetero-oligomerization, functional selectivity, or multiple G protein interactions—phenomena that could explain a receptor's ability to induce unique or multiple signaling responses.

With regard to neurobiological function, evidence suggests that the D_5 receptor is probably the D_1-like receptor subtype

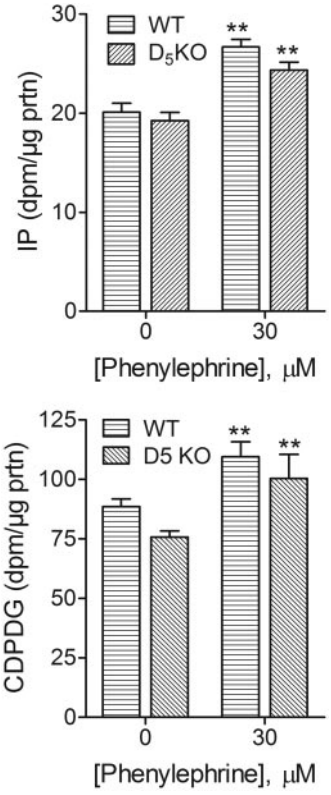

Fig. 3. Phenylephrine-stimulated inositol phosphate and CDP-diacylglycerol accumulation. Experiments were conducted as described in Fig. 1 using frontal cortex tissues from WT and D_5KO mice. Each bar is the mean ± S.E.M. (n = 6). ++, $p < 0.01$ compared with the respective wild-type or knockout control (0 μM phenylephrine).

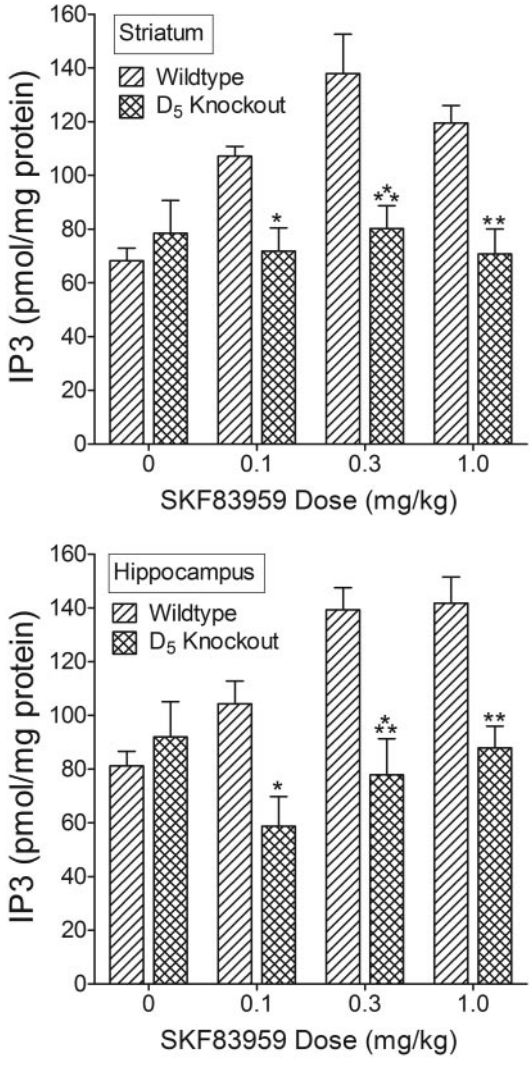

Fig. 4. Effects of D_5 receptor deletion on SKF83959-induced formation of endogenous inositol-1,4,5-trisphosphate. Wild-type and D_5 receptor knockout mice were injected with the indicated dosages of SKF83959 dissolved in 5 ml/kg saline. After 20 min, the animals were killed, and the brains were quickly dissected and flash-frozen for subsequent assay of inositol-1,4,5-trisphosphate using the radioreceptor binding technique. Tissues from each hemisphere were processed separately and the data pooled for statistical analysis. Each bar represents the mean ± S.E.M. (n = 6 hemispheres). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, post hoc Bonferroni comparisons of D_5KO versus WT responses at each drug concentration.

that mediates a range of dopamine's effects, including the regulation of peripheral blood pressure (Hollon et al., 2002), enhanced acetylcholine release in the hippocampus (Hersi et al., 2000), stimulation of pituitary prolactin secretion (Saller and Salama, 1986), and modulation of mucosal vulnerability to psychosomatic ulcerogenic insults (Hunyady et al., 2001). At the behavioral level, congenic D_5 receptor mutant mice show marked reductions in grooming, a characteristic D_1-like dopaminergic response that is nevertheless not cyclase-mediated (O'Sullivan et al., 2005). Moreover, orofacial movement topographies inducible in naive animals by SKF83959 (which does not stimulate adenylyl cyclase) are severely disrupted in congenic D_5 mutant mice (Tomiyama et al., 2006). Hence, the D_5 receptor probably regulates a defined subset of physiological dopaminergic responses, as was believed previously for the dopamine-linked PI signaling response (Undie et al., 2000). Our present observations, therefore, offer potential new clues toward clarifying the downstream intracellular pathways that define the neurochemical and behavioral phenotype of the D_5 dopamine receptor.

Acknowledgments

We thank David Cabrera for technical assistance.

References

Baufreton J, Garret M, Rivera A, de la Calle A, Gonon F, Dufy B, Bioulac B, and Taupignon A (2003) D5 (not D1) Dopamine receptors potentiate burst-firing in neurons of the subthalamic nucleus by modulating an L-type calcium conductance. *J Neurosci* **23:**816–825.

Centonze D, Grande C, Usiello A, Gubellini P, Erbs E, Martin AB, Pisani A, Tognazzi N, Bernardi G, Moratalla R, et al. (2003) Receptor subtypes involved in the presynaptic and postsynaptic actions of dopamine on striatal interneurons. *J Neurosci* **23:**6245–6254.

Ciliax BJ, Nash N, Heilman C, Sunahara R, Hartney A, Tiberi M, Rye DB, Caron MG, Niznik HB, and Levey AI (2000) Dopamine D_5 receptor immunolocalization in rat and monkey brain. *Synapse* **37:**125–145.

Civelli O, Bunzow JR, and Grandy DK (1993) Molecular diversity of the dopamine receptors. *Annu Rev Pharmacol Toxicol* **33:**281–307.

Cook SJ, Palmer S, Plevin R, and Wakelam MJO (1990) Mass measurement of inositol 1,4,5-trisphosphate and Sn-1,2-diacylglycerol in bombesin-stimulated Swiss 3T3 mouse fibroblasts. *Biochem J* **265:**617–620.

Dwivedi Y, Rizavi HS, Rao JS, and Pandey GN (2000) Modifications in the phosphoinositide signaling pathway by adrenal glucocorticoids in rat brain: focus on phosphoinositide-specific phospholipase C and inositol 1,4,5-trisphosphate. *J Pharmacol Exp Ther* **295:**244–254.

Felder CC, Jose PA, and Axelrod J (1989) The dopamine-1 agonist SKF82526 stimulates phospholipase-C activity independent of adenylate cyclase. *J Pharmacol Exp Ther* **248:**171–175.

Friedman E, Jin LQ, Cai GP, Hollon TR, Drago J, Sibley DR, and Wang HY (1997) D1-like dopaminergic activation of phosphoinositide hydrolysis is independent of D1A dopamine receptors: evidence from D1A knockout mice. *Mol Pharmacol* **51:**6–11.

Gnanalingham KK, Hunter AJ, Jenner P, and Marsden CD (1995) Stimulation of adenylate cyclase activity by benzazepine D-1 dopamine agonists with varying efficacies in the 6-hydroxydopamine lesioned rat–relationship to circling behaviour. *Biochem Pharmacol* **49:**1185–1193.

Hermans E (2003) Biochemical and pharmacological control of the multiplicity of coupling at G-protein-coupled receptors. *Pharmacol Ther* **99:**25–44.

Hersi AI, Kitaichi K, Srivastava LK, Gaudreau P, and Quirion R (2000) Dopamine D-5 receptor modulates hippocampal acetylcholine release. *Brain Res Mol Brain Res* **76:**336–340.

Hollon TR, Bek MJ, Lachowicz JE, Ariano MA, Mezey E, Ramachandran R, Wersinger SR, Soares-da-Silva P, Liu ZF, Grinberg A, et al. (2002) Mice lacking D5 dopamine receptors have increased sympathetic tone and are hypertensive. *J Neurosci* **22:**10801–10810.

Hunyady B, Palkovits M, Mózsik G, Molnár J, Fehér K, Tóth Z, Zólyomi A, Szalayova I, Key S, Sibley DR, et al. (2001) Susceptibility of dopamine D5 receptor targeted mice to cysteamine. *J Physiol Paris* **95:**147–151.

Jin LQ, Goswami S, Cai G, Zhen X, and Friedman E (2003) SKF83959 selectively regulates phosphatidylinositol-linked D1 dopamine receptors in rat brain. *J Neurochem* **85:**378–386.

Karpa KD, Lidow MS, Pickering MT, Levenson R, and Bergson C (1999) N-linked glycosylation is required for plasma membrane localization of D5, but not D1, dopamine receptors in transfected mammalian cells. *Mol Pharmacol* **56:**1071–1078.

Lachowicz JE and Sibley DR (1997) Molecular characteristics of mammalian dopamine receptors. *Pharmacol Toxicol* **81:**105–113.

Le Crom S, Prou D, and Vernier P (2002) Autocrine activation of adenosine A1 receptors blocks D1A but not D1B dopamine receptor desensitization. *J Neurochem* **82:**1549–1552.

Lezcano N and Bergson C (2002) D1/D5 dopamine receptors stimulate intracellular calcium release in primary cultures of neocortical and hippocampal neurons. *J Neurophysiol* **87:**2167–2175.

Mahan LC, Burch RM, Monsma FJ Jr, and Sibley DR (1990) Expression of striatal D1 dopamine receptors coupled to inositol phosphate production and Ca^{2+} mobilization in *Xenopus* oocytes. *Proc Natl Acad Sci U S A* **87:**2196–2200.

Meador-Woodruff JH, Mansour A, Grandy DK, Damask SP, Civelli O, and Watson SJ Jr (1992) Distribution of D5 dopamine receptor MRNA in rat brain. *Neurosci Lett* **145:**209–212.

Monsma FJ Jr, Mahan LC, McVittie LD, Gerfen CR, and Sibley DR (1990) Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. *Proc Natl Acad Sci U S A* **87:**6723–6727.

Montague DM, Striplin CD, Overcash JS, Drago J, Lawler CP, and Mailman RB (2001) Quantification of D1B(D5) receptors in dopamine D1A receptor-deficient mice. *Synapse* **39:**319–322.

Neve KA, Seamans JK, and Trantham-Davidson H (2004) Dopamine receptor signaling. *J Recept Signal Transduct Res* **24:**165–205.

O'Sullivan GJ, Kinsella A, Sibley DR, Tighe O, Croke DT, and Waddington JL (2005) Ethological resolution of behavioural topography and D1-like versus D2-like agonist responses in congenic D5 dopamine receptor mutants: identification of D5: D2-like interactions. *Synapse* **55:**201–211.

Panchalingam S and Undie AS (2001) SKF83959 exhibits biochemical agonism by stimulating phosphoinositide hydrolysis and [^{35}S]GTPgS binding in rat and monkey brain. *Neuropharmacology* **40:**826–837.

Panchalingam S and Undie AS (2005) Physicochemical modulation of agonist-induced [35S]GTPgammaS binding: implications for coexistence of multiple functional conformations of dopamine D1-like receptors. *J Recept Signal Transduct Res* **25:**125–146.

Paspalas CD and Goldman-Rakic PS (2004) Microdomains for dopamine volume neurotransmission in primate prefrontal cortex. *J Neurosci* **24:**5292–5300.

Rivera A, Alberti I, Martín AB, Narváez JA, de la Calle A, and Moratalla R (2002) Molecular phenotype of rat striatal neurons expressing the dopamine D5 receptor subtype. *Eur J Neurosci* **16:**2049–2058.

Rosengarten H and Friedhoff AJ (1998) A phosphoinositide-linked dopamine D1 receptor mediates repetitive jaw movements in rats. *Biol Psychiatry* **44:**1178–1184.

Saller CF and Salama AI (1986) D-1 dopamine receptor stimulation elevates plasma prolactin levels. *Eur J Pharmacol* **122:**139–142.

Sidhu A and Niznik HB (2000) Coupling of dopamine receptor subtypes to multiple and diverse G proteins. *Int J Dev Neurosci* **18:**669–677.

Tiberi M, Jarvie KR, Silvia C, Falardeau P, Gingrich JA, Godinot N, Bertrand L, Yang-Feng TL, Fremeau RT Jr, and Caron MG (1991) Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. *Proc Natl Acad Sci U S A* **88:**7491–7495.

Tomiyama K, Makihara Y, Yamamoto H, O'Sullivan G, Nally RE, Tighe O, Kinsella A, Fienberg AA, Grandy DK, Sibley DR, et al. (2006) Disruption of orofacial movement topographies in congenic mutants with dopamine D5 but not D4 receptor or DARPP-32 transduction 'knockout'. *Eur Neuropsychopharmacol* **16:**437–445.

Undie AS (1999) Relationship between dopamine agonist stimulation of inositol phosphate formation and cytidine diphosphate-diacylglycerol accumulation in brain slices. *Brain Res* **816:**286–294.

Undie AS, Berki AC, and Beardsley K (2000) Dopaminergic behaviors and signal transduction mediated through adenylate cyclase and phospholipase C pathways. *Neuropharmacology* **39:**75–87.

Undie AS and Friedman E (1990) Stimulation of a dopamine D1 receptor enhances inositol phosphates formation in rat brain. *J Pharmacol Exp Ther* **253:**987–992.

Undie AS and Friedman E (1992) Selective dopaminergic mechanism of dopamine and SKF38393 stimulation of inositol phosphate formation in rat brain. *Eur J Pharmacol* **226:**297–302.

Undie AS, Weinstock J, Sarau HM, and Friedman E (1994) Evidence for a distinct D1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. *J Neurochem* **62:**2045–2048.

Yasumoto F, Negishi T, Ishii Y, Kyuwa S, Kuroda Y, and Yoshikawa Y (2004) Endogenous dopamine maintains synchronous oscillation of intracellular calcium in primary cultured-mouse midbrain neurons. *Cell Mol Neurobiol* **24:**51–61.

Zhen X, Uryu K, Wang HY, and Friedman E (1998) D1 dopamine receptor agonists mediate activation of P38 mitogen-activated protein kinase and C-Jun amino-terminal kinase by a protein kinase A-dependent mechanism in SK-N-MC human neuroblastoma cells. *Mol Pharmacol* **54:**453–458.

Address correspondence to: Dr. Ashiwel S. Undieh, Laboratory of Integrative Neuropharmacology, Department of Pharmaceutical Sciences, Thomas Jefferson University School of Pharmacy, 130 South 9th Street, Suite 1540, Philadelphia, PA 19107. E-mail: ashiwel.undieh@jefferson.edu