Functional Metabotropic Glutamate Receptors on Nuclei from Brain and Primary Cultured Striatal Neurons

ROLE OF TRANSPORTERS IN DELIVERING LIGAND*

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G-protein-coupled receptors are well known for converting an extracellular signal into an intracellular response. Here we showed that the metabotropic glutamate receptor 5 (mGlu5) plays a dynamic intracellular role in signal transduction. Activation of endogenously expressed mGlu5 on striatal nuclear membranes leads to rapid, sustained calcium (Ca2+) responses within the nucleoplasm that can be blocked by receptor-specific antagonists. Extracellular ligands such as glutamate and quisqualate reach nuclear receptors via both sodium-dependent transporters and cysteine glutamate exchangers. Inhibition of either transport system blocks radiolabeled agonist uptake as well as agonist-induced nuclear Ca2+ changes. Impermeable antagonists like LY393503 and LY367386 not only blocked [3H]quisqualate binding but also prevented nontransported agonists such as (RS)-3,5-dihydroxyphenylglycine from inducing intracellular Ca2+ changes in heterologous cells. In contrast, neither LY compound prevented quisqualate or glutamate from activating intracellular receptors leading to Ca2+ responses. Inasmuch as Ca2+ can enter the nucleoplasm via the nuclear pore complex or from the nuclear lumen, the presence of nuclear mGlu5 receptors appeared to amplify the latter process generating a faster nuclear response in heterologous cells. In isolated striatal nuclei, nuclear receptor activation results in the de novo appearance of phosphorylated CREB protein. Thus, activation of nuclear mGlu5 receptors initiates a signaling cascade that is known to alter gene transcription and regulate many paradigms of synaptic plasticity. These studies demonstrated that mGlu5 receptors play a dynamic role in signaling both on and off the plasma membrane.

The structure and function of G-protein-coupled receptors have received intense scrutiny over the past decades. These studies point to a dynamic environment in which receptors are not static but rather move on and off the plasma membrane according to environmental stimuli, specific targeting information, protein-protein interactions, etc. In this model, intracellular receptors are considered transitional, i.e. receptors that are either ready to be inserted into the plasma membrane or that have just been sequestered from such a site. Emerging data, however, suggest that some intracellular receptors may have intracellular functions as well. For example, a number of G-protein-coupled receptors such as the opsin, angiotensin AT1 and ATII, bradykinin B2, and lysophosphatidate LP1 receptors have been localized within the nucleoplasm itself (1–3). In contrast, prostaglandin E2 receptors have been found on the nuclear envelope together with their ligand-generating enzymes (4, 5). Similarly, endothelin receptors A and B are also localized to the perinuclear region of cardiac ventricular myocytes where they mediate nuclear Ca2+ levels and activate nuclear protein kinases (6). Finally, we have shown that the metabotropic glutamate receptor, mGlu5,1 can be expressed on nuclear membranes where it can couple with endogenous signaling components to induce changes in nuclear Ca2+ (7). Because most studies investigating the properties of nuclear receptors have been performed in heterologous cell types with overexpressed receptors, the question arises as to whether such phenomena are physiologically relevant and, in the case of mGlu5, how a ligand such as glutamate has access to this receptor.

1 The abbreviations used are: mGlu5, metabotropic glutamate receptor 5; CREB, cAMP-response element-binding protein; xCT, cystine/glutamate exchanger; EAAAT, excitatory amino acid transporter; P1, postnatal day 1; DIV, days in vitro; CPCCOEt, 7-(hydroxyimino)cyclopropan[b]chromen-1a-carboxylate ethyl ester; MPEP, 2-methyl-6-(phenylethenyl)-pyridine; TBOA, threo-2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; HEK, human embryonic kidney cells; GABA, γ-aminobutyric acid; NMDG, N-methyl-D-glucamine; IP3, inositol 1,4,5-trisphosphate; HA, hemagglutinin.

Numerous morphological and physiological studies have suggested that mGlu5 receptors are enriched in the striatum where they are expressed on postsynaptic neurons, subsets of interneurons, as well as at presynaptic sites (14). Both at the light and electron microscopic level, large amounts of mGlu5 are intracellularly localized (40–70%) where receptors are predominantly localized to the perinuclear region of cardiac ventricular myocytes where they mediate nuclear Ca2+ levels and activate nuclear protein kinases (6). Finally, we have shown that the metabotropic glutamate receptor, mGlu5,1 can be expressed on nuclear membranes where it can couple with endogenous signaling components to induce changes in nuclear Ca2+ (7). Because most studies investigating the properties of nuclear receptors have been performed in heterologous cell types with overexpressed receptors, the question arises as to whether such phenomena are physiologically relevant and, in the case of mGlu5, how a ligand such as glutamate has access to this receptor.

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associated with the endoplasmic reticulum (ER) or nuclear membranes (14). Immunofluorescent staining of embryonic or neonatal striatal cultures shows widespread mGlu5 expression; agonist exposure elicits an intracellular rise in \( \text{Ca}^{2+} \) by a brief increase followed by a sustained plateau. This response can be blocked by application of the mGlu5-specific antagonist MPEP (15). Moreover, activation of striatal mGlu5 receptors leads to phosphorylation of the CREB transcription factor and subsequent activation of several immediate early genes (16). Thus, neonatal striatal cultures seem to be an ideal preparation to test the notion that endogenous mGlu5 receptors are expressed on nuclear membranes where they are coupled to signaling mechanisms within the nuclear membrane.

For intracellular mGlu5 receptors to be of functional relevance, there must be a way to activate them. By using tagged receptor molecules, we have shown previously that the mGlu5 receptor topology is oriented such that ligand binding domains are within the lumen of the nuclear envelope. Thus, a ligand source from the extracellular milieu must traverse both the plasma membrane as well as an intracellular membrane. In theory, some combination of plasma membrane and intracellular transporter could transfer glutamate into the nuclear lumen. For example, there are five sodium-dependent glutamate transporter proteins that are present on the plasma membrane of glial and neuronal cells as well as many peripheral tissues (17). Many other glutamate carriers exist, however, including the cystine-glutamate exchanger. Ubiquitously expressed throughout the body, the exchanger is a sodium-independent, anionic amino acid transporter composed of two separate proteins, xCT and 4F2 (18). The former confers substrate specificity, and the latter is common to many amino acid transporters. Because the mGlu5 agonist, quisqualate, is a substrate for the xCT exchanger (19), it too is a plausible candidate for intracellular ligand delivery.

Here we show that mGlu5 receptors are expressed on the cell surface and intracellular membranes of neonatal striatal neurons. Activation of nuclear receptors leads to increased nuclear-ooplasmic \( \text{Ca}^{2+} \), which can be blocked by receptor-specific antagonists. Ligand delivery is via both sodium-dependent and -independent mechanisms. Impermeable antagonists can block cellular responses to agonists that are not transported into cells but cannot block those that are. Finally, agonist-induced nuclear receptor activation leads to phosphorylation of CREB. Taken together, these data challenge existing paradigms by suggesting a new mode of intracellular signal transduction mediated in situ by G-protein-coupled receptors.

**EXPERIMENTAL PROCEDURES**

**Materials**—2-Amino-2-(4-carboxyphenyl)-3-(9H-thioxanthan-9-yl) propanoic acid (LY367366) and 2-amino-2-(3-is and trans-carbocyclohexyl)-3-(9H-thioxanthan-9-yl)propanic acid (LY389053) were obtained from Lilly. Quisqualate and (RS)-3,5-dihydroxyphenylglycine (DHPG) were purchased from Torcis Cookson Inc. (Ellisville, MO). Unless otherwise indicated all other chemicals were from Sigma.

**Cell Culture and Immunocytochemistry**—The mGlu5/HEK stable cell line was generated and maintained as described (20). The mGlu5/GABA-B1 chimera (mG5C1) as well as its mutant (mG5C1mut), were kind gifts from Dr. J. P. Pin (Institute of Functional Genomics, CNRS, France). Primary striatal neuronal cultures using neonatal 1-day-old rat pups were prepared and maintained as detailed by Mao and Wang (15). Fixation, blocking, and antibody incubation were as described previously (20). Primary antibodies included affinity-purified polyclonal anti-C-terminal mGlu5 antibody (1:1,300) (21), monoclonal anti-lamin B1 (1:1,000), monoclonal anti-alpha-1K-ATPase. Anti-EAA3 and xCT antibodies (Dr. J. Rothstein, The Johns Hopkins University) were used at dilutions of 1:200 and 1:1,500, respectively. A horseradish peroxidase conjugated with goat anti-rabbit IgG (1:2000; Cell Signaling Technology, Inc.) or anti-mouse IgG (1:2500; Sigma) was used in conjunction with enhanced chemiluminescence (Amersham Biosciences) to detect the signal. Densitometric analyses of mGlu5 proteins were performed by using the Storm 860 Imager (Amersham Biosciences) together with associated software.

**Plasma and Intracellular Ca**\(^{2+}\) and uptake measurements performed on intact cells were done in Krebs-Ringer solution (KRS), containing the following (in mM): 137 NaCl, 5.1 KCl, 0.77 KH\(_2\)PO\(_4\), 0.71 MgSO\(_4\)/H\(_2\)O, 1.0 CaCl\(_2\), 10 H-glucose, and 10 HEPES. To achieve chloride-free conditions (Cl\(^{-}\)-free), the buffer contained the following (in mM): 130 sodium glutamate, 5 potassium glutamate, 1.1 calcium gluconate, 0.77 KH\(_2\)PO\(_4\), 0.71 MgSO\(_4\)/H\(_2\)O, 1.0 glutamic acid, and 10 HEPES. To achieve chloride- and calcium-free conditions (Na\(^{+}\)-Ca\(^{2+}\)-free) the buffer contained the following (in mM): 137.5 choline chloride, 5.36 KCl, 0.77 KH\(_2\)PO\(_4\), 0.71 MgSO\(_4\)/H\(_2\)O, 1.1 CaCl\(_2\), and 10 HEPES. Sodium- and chloride-free solutions (Na\(^{+}\)-Cl\(^{-}\)-free) were achieved by using a buffer containing the following (in mM): 274 N-methyl-d-glucamine, 5 potassium glutamate, 1.1 calcium gluconate, 0.77 KH\(_2\)PO\(_4\), 0.71 MgSO\(_4\)/H\(_2\)O, 1.0 glutamic acid, and 10 HEPES (pH 7.4 adjusted with 50% glucose). Flurescent Measurements of Intracellular Ca\(^{2+}\) in Intracellular buffer conditions. Intracellular buffers contained the following (in mM): 125 KCl, 2 KH\(_2\)PO\(_4\), 2 MgCl\(_2\), 0.3 CaCl\(_2\), 10 H-glucose, 1 ATP, and 40 HEPES (pH 7.0).

**H-Labeled Agonist Uptake**—[\(^{3}H\)]Quisqualate (26.0 Ci/mmole) and \( [\text{H}] \)glutamate (43.0 Ci/mmole) were obtained from Amersham Biosciences. In some cases, radiolabeled agonists (3.25 μCi/ml [\(^{3}H\)]quisqualate or 4.08 μCi/ml [\(^{3}H\)]glutamate) were added together with an unlabeled drug to achieve a total quisqualate concentration of 120 and 500 nm, 1 or 10 μM, or a total concentration of 10 μM or 1 mM glutamate. The mGlu5/HEK stable cell line (5 × 10\(^5\) cells/well) were cultured at 37 °C for 3 or 9 days, respectively. Before use, purified nuclei were washed three times in the appropriate buffer and then incubated at 37 °C in the absence of various concentrations of t-cystine or TBOA for 15 min before adding labeled agonist. Uptake was terminated after 15 min. Samples were rapidly rinsed three times with ice-cold phosphate-buffered saline, solubilized in 150 μl of 1% Triton X-100/phosphate-buffered saline, and then analyzed by liquid scintillation.

**[\(^{3}H\)]Quisqualate Binding Assay**—Plasma membrane fractions were prepared as described above from mGlu5-stable HEK cells. Membranes were subsequently washed three times in 2 mM EDTA, 2 mM HEPES (pH 7.5), with protease inhibitors followed by centrifugation at 17,000 × g for 35 min. The final pellets were resuspended in buffer containing 40 mM HEPES (pH 7.5), 2.5 mM Ca\(^{2+}\), and protease inhibitors. Binding was performed as described (20).

**Fluorescent Measurements of Intracellular Ca\(^{2+}\)**—For whole cell measurements, dissociated striatal neurons were grown on 35-mm dishes with glass grids (1.2 × 10\(^5\) cells/grid), washed with Neurobasal medium (NB, Invitrogen), and incubated with 5 μM Oregon Green 488 BAPTA-AM, 0.000% pluronic acid (Molecular Probes, Eugene, OR) in NB for 30 min at 37 °C. Cells were washed three times with NB and observed using a laser confocal microscope (Olympus BX 50W) with an AxiosCam HR camera (Zeiss, Thornwood, NY) as the imaging objectives. The real-time images were captured by an Olympus Fluoview FXV confocal laser scanning system using Fluoview 2.0 acquisition software. Images were converted to TIF files for processing with MetaMorph (version 5.0.7) Professional Image Analysis software, produced by Universal Imaging. Drugs at 100× concentrations were added to the side of the dish and allowed to diffuse over the cells at room temperature. Quisqualate was added at a final concentration of 10 μM together with 5 μM OYK35655
Intracellular mGlu5 Receptors Mobilize Nuclear Calcium

INTRACELLULAR mGlu5 RECEPTORS MOBILIZE NUCLEAR Ca2+

FIG. 1. Striatal mGlu5 receptors are expressed on cell bodies and nuclear membranes where they mediate intracellular Ca2+ changes. A, DIV9 striatal culture showing co-localization of mGlu5 receptor immunoreactivity (red) together with anti-GABA staining (green). B, DIV9 striatal neurons showing mGlu5 immunoreactivity co-localized with the inner nuclear marker, lamin B2. C, transmitted light image of post hoc identified mGlu5-positive cell (not shown) indicating regions measured in D. D, nuceloplasmic [Ca2+] (red circle, red line) and cytoplasmic [Ca2+] (blue circle, blue line). Quisqualate (Quis) (10 μM) and MPEP (1 μM) were added as indicated. Because quisqualate would also activate AMPA channels and mGlu1 receptors, it was bath-applied in the presence of 5 μM GYKI53655, an AMPA antagonist as well as CPCCOEt, an mGlu1 antagonist (20 μM). Agonist application under these conditions led to a rise in calcium consisting of two phases, an initial rapid rise followed by a sustained elevation. This response was terminated by the addition of the highly specific mGlu5 antagonist MPEP. E, compiled data from experiments on post hoc identified mGlu5-positive cells. Taken together, these data are consistent with the idea that mGlu5 is abundantly expressed in embryonic or neonatal striatal neurons (15), we established striatal cultures from P1 rat pups. The vast majority of neurons present in these cultures was GABA-ergic as determined by anti-GABA antibodies. Typically, about half of the GABA-ergic neurons were mGlu5-positive (Fig. 1A). Wells stained on the 9th day in vitro (DIV) showed numerous immunofluorescent neurons in which mGlu5 could be visualized on cell processes, intracellularly, and on the nuclear membrane (Fig. 1B). With increased length of time in culture, receptor levels appeared to increase particularly on the plasma membrane and cell processes, although nuclear receptors were always detectable even after 2–3 weeks in culture (not shown). No mGlu1a receptor staining was observed at any age in culture in agreement with reports that mGlu1b is the predominant subtype expressed (Ref. 22; data not shown). Despite the presence of glia, no pronounced mGlu5 staining of this cell type was observed.

To test the hypothesis that activation of mGlu5 receptors expressed on nuclear striatal membranes leads to changes in nuclear Ca2+, DIV 9 cultures grown on glass coverslips were loaded with the Ca2+ indicator, Oregon Green BAPTA-AM, and subsequently treated with the group 1 agonist, quisqualate (10 μM). Because quisqualate would also activate AMPA channels as well as mGlu1 receptors, it was bath-applied in the presence of 5 μM GYKI53655, an AMPA antagonist, and CPCCOEt, an mGlu1 antagonist (20 μM). Agonist application under these conditions led to a rise in Ca2+ in both the cytoplasm as well as the nucleus consisting of two phases, an initial rapid rise followed by a sustained elevation. Both sets of responses were terminated by the addition of the highly specific mGlu5 antagonist, MPEP (1 μM; Fig. 1D). Fig. 1E represents compiled data from multiple experiments on post hoc identified mGlu5-positive cells. Taken together, these data are consistent with nuclear mGlu5 receptors coupling to G-proteins localized on nuclear membranes, but they do not rule out the possibility that the observed rises in Ca2+ are because of other indirect causes.

RESULTS

Agonist Treatment Triggers Cytosolic and Nuclear Ca2+ Responses in Striatal Neurons Expressing mGlu5 Receptors—As mGlu5 is abundantly expressed in embryonic or neonatal striatal neurons (15), we established striatal cultures from P1 rat pups. The vast majority of neurons present in these cultures was GABA-ergic as determined by anti-GABA antibodies. Typically, about half of the GABA-ergic neurons were mGlu5-positive (Fig. 1A). Wells stained on the 9th day in vitro (DIV) showed numerous immunofluorescent neurons in which mGlu5 could be visualized on cell processes, intracellularly, and on the nuclear membrane (Fig. 1B). With increased length of time in culture, receptor levels appeared to increase particularly on the plasma membrane and cell processes, although nuclear receptors were always detectable even after 2–3 weeks in culture (not shown). No mGlu1a receptor staining was observed at any age in culture in agreement with reports that mGlu1b is the predominant subtype expressed (Ref. 22; data not shown). Despite the presence of glia, no pronounced mGlu5 staining of this cell type was observed.

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Agonist Treatment Directly Triggers Nuclear Ca2+ Responses—To assess more directly nuclear mGlu5 receptor function, isolated nuclei were prepared from DIV 9 cultures. Nuclei were resuspended in intracellular medium, and Oregon Green BAPTA-AM was allowed to accumulate while nuclei were at-
agonist carbachol did not elicit a nuclear Ca$^{2+}$ rise in the course of nuclear preparation. The muscarinic Presumably nonresponding mGlu5-positive nuclei were damaged in the course of nuclear preparation. The mGlu5-positive nuclei never responded to quisqualate (11:36), whereas non-mGlu5-positive nuclei that post hoc stained for mGlu5 responded to agonist treatment (11:36), whereas non-mGlu5-positive nuclei were compiled from multiple nuclei at 300 s (Fig. 2B). MPEP (1 μM) blocked this response. To confirm mGlu5 receptor presence on responding nuclei, coverslips were fixed and processed for mGlu5 immunoreactivity immediately following imaging (Fig. 2A, lower panel). One hundred percent of responsive cells were mGlu5-positive. Data were compiled from multiple nuclei at 300 s (Fig. 2C). Approximately 30% of nuclei that post hoc stained for mGlu5 responded to agonist treatment (11:36), whereas non-mGlu5-positive nuclei never responded to quisqualate (n = 45). Presumably nonresponding mGlu5-positive nuclei were damaged in the course of nuclear preparation. The muscarinic agonist carbachol did not elicit a nuclear Ca$^{2+}$ response nor did other agonists for other endogenous receptors such as Substance P, adenosine A1 and A2, or a1 adrenergic receptors (not shown).

Because of the difficulty of preparation and low yield of nuclei from dissociated cultures, we developed an acute isolation procedure directly from brain tissue. Striata collected at postnatal (P) days 1, 3, and 10 were pooled and fractionated, and the nuclei were purified. Western blotting was used to determine the level of nuclear receptors present in striatal tissues. The mGlu5 receptor was clearly expressed in both nuclear and plasma membrane fractions as indicated by the membrane-specific markers lamin B2 and Na$^+$,K$^+$-ATPase, respectively (Fig. 3A). Levels of both cell surface and nuclear receptors increased throughout this time period consistent with previous reports (P10 versus P1, 165% increase, p < 0.02) (21). Unlike heterologous cell types and/or P3 forebrain tissue (7), striatal nuclear receptors migrated slightly slower than plasma membrane mGlu5 (Fig. 3A).

Purified nuclei were loaded with the Ca$^{2+}$ fluorophore and assessed for agonist-induced changes in fluorescence. In 80% of the nuclei measured, slow, gradual Ca$^{2+}$ rises were apparent at all developmental time points (Fig. 3, B and C). These were most pronounced at P10. Approximately 10% of the time an agonist-induced oscillatory response was observed that could be blocked by MPEP (Fig. 3, B and C). Results were essentially the same for nuclei prepared from either rat or murine striata (not shown). Taken together, these data demonstrate that activation of endogenous nuclear mGlu5 receptors increase nuclear Ca$^{2+}$ levels, establishing the role of nuclear mGlu5 receptors in a native, physiological setting.

**xCT Mediates Nuclear mGlu5 Receptor Activation in Heterologous Cell Types**—Does glutamate activate these intracellular receptors, and if so, how does it do so? To test the idea that an mGlu5 agonist could access intracellular receptors, we used two basic approaches, localization of bath-applied drug using anti-ligand antibodies as well as uptake of radiolabeled agonist using standard paradigms. Because antibodies directed against quisqualate have been shown to specifically stain quisqualate-treated neurons (23), they provided a means by which quisqualate uptake could be visualized in treated cells. Specifically, agonist was rapidly taken up by HEK cells where it was detected within the cytoplasm and the nucleus within a minute of application (Fig. 4A). Thus quisqualate rapidly gets into the cell, but how is it doing so?

To test directly whether HEK cells and/or nuclei could take up quisqualate, both were treated with radiolabeled ligand in the presence and absence of transport or exchange inhibitors. Although our previous results in HEK cells suggested that sodium-dependent plasma membrane transporters did not play a major role in this process, we tested their contribution as well as the cystine/glutamate exchanger that is widely expressed in the central nervous system as well as the periphery (18). [$^{[3H]}$Quisqualate was readily taken up by intact HEK cells as well as nuclei (Fig. 4, B and C). By using whole cells in extracellular sodium-free buffer conditions (choline substitution), ~20% of total quisqualate uptake was blocked, suggesting that sodium-dependent plasma membrane transporters play a small but significant role in transporting ligand across the plasma membrane (Fig. 4B). In contrast, inhibition of xCT with 0.4 mM cystine blocked 50% of uptake in whole cells or isolated nuclei (Fig. 4C). Most interestingly, sodium-free conditions (i.e. intracellular buffer conditions) had no effect on nuclear uptake (Fig. 4C), demonstrating that luminal import of mGlu5 agonist is via xCT. Moreover, application of l-cysteine in sodium-free buffer further reduced quisqualate uptake in whole cells but not in nuclear membranes (Fig. 4, B and C). The IC$_{50}$ for cystine in whole cells was 146 ± 45 μM. More importantly, levels of cysteine up to 0.4 mM did not affect receptor binding (not shown). Finally, application of 0.4 mM l-cysteine to quisqualate-induced nuclear oscillations dramatically blocked further response (Fig. 4D). Taken together, this set of data suggests that mGlu5 ligands can be taken up by at least two transport mechanisms, the sodium-dependent plasma membrane transporter as well as the xCT exchanger.

xCT and Sodium-dependent Transporters Mediate Nuclear mGlu5 Receptor Activation in Striatal Neurons—As in heterologous cultures, rapid uptake of quisqualate can be visualized in rat striatal cultures using the anti-quisqualate antibody (Fig. 5A). Within a minute of agonist application, it can be detected within neuronal cell bodies, nuclei, and processes. Consistent with the notion that a specific uptake process is required, only some neurons appeared to take quisqualate up (Fig. 5A). Even
Fig. 3. P1, P3, and P10 acutely isolated striatal nuclei express mGlu5 and exhibit agonist-mediated Ca\(^{2+}\) changes. A. subcellular fractionation of P1, P3, or P10 striata shows that mGlu5 receptor can be detected in fractions containing both the nuclear (N) and plasma membranes (PM). Thirty micrograms of protein from each fraction were separated on reducing SDS gels and transferred to nylon membranes. The same blot was sequentially probed with antibodies against mGlu5, the inner nuclear marker, lamin B\(_2\), and the plasma membrane marker, Na\(^+\),K\(^+\)-ATPase. B, quisqualate (Quis)-mediated (10 \(\mu\)M) representative traces from nuclei prepared at indicated developmental time points. Quisqualate application resulted in Ca\(^{2+}\) rises in 80–90% of the nuclei measured that continued to rise across the period examined. MPEP (1 \(\mu\)M) blocked this response. C, compiled data of \(\Delta F/F_o\) (%) from the indicated number of cells and time points. All cells were post hoc fixed, stained for mGlu5, and field re-located to ensure specificity of response. \(a = p < 10^{-4}\) when compared with base-line Ca\(^{2+}\) levels; \(b = p < 0.02\) when compared with P1 and P3 levels; \(c = p < 0.005\) when compared with P1 and P3 levels. D, between 10 and 20% of acutely isolated striatal nuclei exhibited agonist-induced oscillatory responses. 1st panel, transmitted light image of selected nucleus; 2nd panel, confocal images of Oregon Green BAPTA loaded acutely dissociated striatal nuclei treated at the indicated times (seconds) with 10 \(\mu\)M quisqualate (Quis) or 1 \(\mu\)M MPEP. Bar at right of last panel represents \(\Delta F/F_o\) as a pseudo color scale with red being the highest. Times correspond to those for traces in E, where oscillations are represented as the fractional change in fluorescence relative to the basal value. D, final panel, mGlu5 receptor and lamin B\(_2\) staining of selected nucleus following drug treatment and post hoc field relocation.

at time points as long as 1 h only 30–40% of the cells are labeled (not shown). Moreover, uptake is dependent upon specific ionic conditions because no quisqualate could be detected in sodium/chloride-free buffers (NMDF; Fig. 5A).

To determine whether known members of the sodium-depend-ent transporters and/or the xCT exchanger were expressed in P10 striatal tissue, total RNA was prepared and used for reverse transcription-PCR. Transcript-specific primers revealed the presence of EAAT1–4 and the xCT exchanger; EAAT5 was not expressed (Fig. 5B). Antibodies directed against the neuronal EAAT3 revealed that in stratal neurons EAAT3 appears highly expressed on cell bodies and processes as well as on the nuclear membrane where it co-localizes with lamin B\(_2\) (Fig. 5C). Finally, Western blotting of P3-fractionated tissue using anti-EAAT3 or anti-xCT antibodies detected proteins within the nuclear fraction of the appropriate size (Fig. 5D). Thus both EAAT3 and xCT are possible nuclear transport candidates.

To test directly whether striatal cells and/or nuclei could take up quisqualate, both were treated with radiolabeled ligand in the presence and absence of transport or exchange inhibitors. Sodium-dependent transporter activity accounted for 20–35% of quisqualate uptake across a wide range of concentrations, whereas chloride-dependent xCT transport, as de-termined by gluconate substitution (or l-cystine addition, not shown), varied from 15 to 60% depending upon the quisqualate concentration (Fig. 5E). Sodium- and chloride-free conditions reduced quisqualate uptake by 90% (Fig. 5E). The IC\(_{50}\) for l-cystine for \([\text{H}]\)quisqualate plasma membrane transport in sodium-free buffer conditions was 112 ± 29 \(\mu\)M. The IC\(_{50}\) for three-benzoxoxyaspartate (TBOA), a potent blocker of all EAAT subtypes, in sodium gluconate buffer (Cl\(^-\)-free) was 0.92 ± 0.27 \(\mu\)M (Fig. 5F). Similar results were observed for \([\text{H}]\)glutamate as the endogenous ligand (Fig. 5G). In contrast to quisqualate uptake, however, sodium-dependent transporters took up a larger percentage of the natural ligand glutamate. Thus both xCT- and/or EAAT-mediated activity allow for intracellular glutamate uptake.

To determine what mediates glutamate uptake into the nuclear lumen, acutely isolated striatal nuclei were prepared in intracellular buffer conditions and used for uptake studies. Nuclear \([\text{H}]\)glutamate uptake exhibited two components, an xCT mechanism accounting for about 50–60% as well as another sodium, potassium, and chloride independent process (Fig. 6A). Quisqualate uptake via the xCT exchanger could be blocked by as little as 50 \(\mu\)M l-cystine or chloride-free buffer conditions (Fig. 6A, K\(_{gluc}\)). Radiolabeled glutamate uptake could also be inhibited by about 50% in chloride-free conditions (Fig. 6B). Despite the sodium-free nature of the intracellular buffer conditions, 30 \(\mu\)M TBOA significantly blocked 30–40% of \([\text{H}]\)glutamate uptake both alone and in combination with chloride-free buffer conditions; 80% of glutamate uptake could be blocked by NMDG (Fig. 6B).
To determine whether uptake via the xCT exchanger was necessary for mGlu5-mediated nuclear calcium changes, quisqualate-induced calcium influxes were treated with 1-cystine (200 \mu M). This reagent blocked nuclear responses, demonstrating that ligand transport is necessary for receptor function (Fig. 6C).

**Impermeable Agonists and Antagonists Differentially Affect Intracellular Ca\(^{2+}\) Changes**—Recently, Kniazeff et al. (24) reported that an mGlu5/GABAB1 chimera, mG5C1, was unable to be trafficked to the cell surface, whereas mutation of an RRX motif from RSRR to ASAR (mG5C1\_ASA) led to functional receptors on the plasma membrane. To test whether an extracellular ligand could activate the intracellular mG5C1 chimera, we obtained these constructs (Fig. 7A), transfected them into HEK cells, and measured Ca\(^{2+}\) changes in response to glutamate or quisqualate application. In agreement with Kniazeff et al. (24), the mG5C1 chimera was not trafficked to plasma membranes, whereas the mG5C1\_ASA mutant was easily seen on the cell surface (Fig. 7B). Similarly, no Ca\(^{2+}\) response was observed in mG5C1-transfected cells, whereas Ca\(^{2+}\) oscillations were observed in both the cytoplasm and the nucleus in mG5C1\_ASA mutant cells (Fig. 7, C and D). The lack of response in the mG5C1 cells is presumably due to the inability of the chimera to be trafficked out of the ER. This is clearly shown in Fig. 7B, where under permeabilized conditions the HA-tagged mG5C1 staining is concentrated in a perinuclear ER location and is not present on nuclear or cell surface membranes as is the mG5C1\_ASA mutant.

Our model would predict that highly charged agonists that are not taken into the cell via specific transport processes would activate cell surface but not intracellular receptors. Similarly, impermeable or nontransported antagonists would block plasma membrane receptors but not intracellular binding sites. To test these predictions, we assessed the effects of various ligands on mGlu5-stable HEK cell lines. The drugs LY393053 and LY367366 are thought to be impermeable antagonists specific for the group 1 mGlu receptors (25). In cells derived from a Syrian hamster cell line, LY393053 and LY367366 blocked mGlu5-mediated IP\(_3\) responses with IC\(_{50}\) values of 1.6 ± 1.4 and 4 ± 1 \mu M, respectively (25). Using membranes prepared from the mGlu5 HEK cells, LY393053 and LY367366 blocked [\(^3\)H]quisqualate binding with IC\(_{50}\) values of 1.35 ± 0.36 and 4.7 ± 0.07 \mu M, respectively (Fig. 8A). Despite blocking quisqualate binding, neither compound inhibited [\(^3\)H]quisqualate uptake (Fig. 8B) or [\(^3\)H]glutamate uptake (not shown) even at doses as high as 100 \mu M. Of course without a radiolabeled analog, it is difficult to completely rule out the possibility that LY393053 and LY367366 may be taken up by other types of amino acid/carboxylate transport systems. It would appear at least that the EAAs and the xCT are not involved. Similarly, the group 1 agonist DHPG did not affect quisqualate (Fig. 8B) or glutamate uptake either, suggesting that it too is a nontransported ligand.

In mGlu5-stable HEK cells, DHPG (100 \mu M) induced pronounced cytoplasmic Ca\(^{2+}\) oscillations as well as nuclear responses (Fig. 8C). These effects could be blocked by the permeable antagonist, MPEP (7) (Fig. 8C). By themselves, neither LY393053 nor LY367366 (40 \mu M) induced intracellular Ca\(^{2+}\) changes (Fig. 8D), although both completely blocked DHPG-mediated Ca\(^{2+}\) responses (Fig. 8E). These data suggest that DHPG selectively activates plasma membrane mGlu5 receptors that are susceptible to inhibition by the LY antagonists. Although nuclear responses are still seen following DHPG treatment (Fig. 8C), these might be due to Ca\(^{2+}\)-activated Ca\(^{2+}\) release and/or Ca\(^{2+}\) entry via nuclear pore complexes. To test this hypothesis, nuclei derived from the stable mGlu5 cell line were treated with DHPG. In this context DHPG was unable to activate a nuclear Ca\(^{2+}\) response, although glutamate (10 \mu M)
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DISCUSSION

Contrary to the idea that intracellular mGlu5 receptors are nonfunctional and merely constitute an internal reserve of receptors waiting to go to the cell surface, the present results demonstrate that mGlu5 receptors play dynamic intracellular roles in signal transduction. These studies show that in a native, physiological setting, i.e. striatal dissociated cultures and acutely isolated striatal nuclei, activation of nuclear mGlu5 receptors leads to rapid, sustained Ca\(^{2+}\) responses that can be blocked by the mGlu5-specific antagonist MPEP. Current results demonstrate that both sodium-dependent transporters as well as xCT exchangers are involved in moving agonist across both plasma and nuclear membranes. Inhibition of either transport system blocks agonist-induced nuclear Ca\(^{2+}\) changes. In the presence of an impermeable antagonist, transported ligands such as quisqualate or glutamate can still induce intracellular Ca\(^{2+}\) responses, whereas nontransported agonists are ineffectual. Finally, ligand stimulation of nuclear receptors initiates at least one signaling cascade that is known to alter gene transcription and regulates many paradigms of phosphorylation in dissociated striatal cultures as well as in isolated nuclei. Robust up-regulation of phosphorylated CREB was observed within minutes of quisqualate application in either paradigm (Fig. 10). CREB phosphorylation was not seen when nuclei were pretreated with MPEP. Thus direct activation of nuclear receptors initiates signaling cascades within the nucleus.

could (Fig. 8F). These data are consistent with the premise that DHPG is not transported across membranes. In contrast, quisqualate induced cytoplasmic and nuclear Ca\(^{2+}\) oscillations (Fig. 8G) even in the presence of LY393053 or LY367366 (40 \(\mu\)M, Fig. 8H).

To confirm and extend the hypothesis that DHPG initially affects cytoplasmic processes, we increased the scan speed (0.25 s/scan) so that the timing of the cytoplasmic and nuclear events could be distinguished. DHPG treatment increased cytoplasmic Ca\(^{2+}\) \(0.55 \pm 0.24\) s prior to its rise in the nucleus (p < 0.002; Fig. 9, A and C), whereas glutamate- or quisqualate-induced nuclear changes preceded those in the cytoplasm by 0.86 \(\pm 0.12\) s (p < 0.004; Fig. 9, B and C, and data not shown). Thus, glutamate induced a faster nuclear response than did DHPG (Fig. 9C). Overall, DHPG induced significantly greater cytoplasmic than nuclear Ca\(^{2+}\) responses, and the opposite was true with glutamate (Fig. 9D). Taken together, these data suggest that depending upon their ability to cross cellular membranes, agonists and antagonists may have differentiable effects on plasma membrane or intracellular receptors.

Activation of Nuclear mGlu5 Receptors Leads to CREB Phosphorylation in Whole Cells and Isolated Striatal Nuclei—What are the functional consequences of mGlu5 receptor-mediated induction of nuclear Ca\(^{2+}\)? As studies have shown that activation of mGlu5 receptors in vitro and in vivo up-regulates several transcription factors including CREB (16), we tested whether quisqualate increased CREB phosphorylation in dissociated striatal cultures as well as in isolated nuclei. Robust up-regulation of phosphorylated CREB was observed within minutes of quisqualate application in either paradigm (Fig. 10). CREB phosphorylation was not seen when nuclei were pretreated with MPEP. Thus direct activation of nuclear receptors initiates signaling cascades within the nucleus.
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Fig. 6. Sodium-dependent and xCT transporters carry ligand into the nuclear lumen. A, by using intracellular buffer conditions as indicated, about 50–60% of [3H]quisqualate (Quis) (10 μM) uptake into striatal nuclei was via xCT transport as determined by inhibition with cystine (10 or 50 μM) or gluconate substitution; about 40% was because of a sodium-, chloride-independent process (NMDG). B, approximately 50% of [3H]glutamate uptake was inhibited in chloride-free conditions (K+ gluconate (gluc)). TBOA (30 μM) blocked 30–40% of [3H]glutamate uptake both alone and in combination with chloride-free buffer conditions. C, quisqualate-induced calcium responses in isolated nuclei were blocked by the xCT inhibitor l-cystine (200 μM).

Endogenously Expressed Nuclear mGlu5 Receptors Mediate Ca2+ Changes—Previously, we have shown (7) that activation of nuclear mGlu5 receptors can trigger Ca2+ oscillations in both the cytoplasm as well as isolated nuclei of heterologous cell types. Because these studies relied upon overexpression of receptors in HEK cells or neurons, it might be argued that the results were nonphysiological. In order to extend these findings into more physiological systems, a dissociated striatal paradigm was adapted (15) in which mGlu5 receptors could be visualized both at the cell surface as well as on nuclear membranes (Fig. 1). As in the previous studies, isolated nuclei that expressed mGlu5 receptors responded to agonist treatment with rapid, sustained Ca2+ responses (Fig. 2). Most interestingly, when acutely isolated nuclei were prepared from P1 to P10 striata (Fig. 3), other patterns of Ca2+ responses were also observed, including prototypic oscillations of mGlu5. These data are consistent with previous reports documenting mGlu5-induced transient Ca2+ increases with sustained plateaus (10–13). Observed differences in temporal profiles may be due to the cellular context that receptor subtypes are expressed in and/or vary with the level of agonist stimulation. For example, Prothero et al. (12) found that cortical neurons gave oscillatory responses in just a few cells at agonist concentrations ranging from 10 to 25 μM, whereas higher agonist concentrations (100 μM) elicited plateau responses in 80% of the neurons.

Levels of plasma membrane and nuclear mGlu5 receptors increased with time in vivo (P1 to P10), mirroring earlier studies showing that glutamate-stimulated phosphoinositide hydrolysis and mobilization of intracellular Ca2+ are more pronounced in the immature brain. Differential patterns of Ca2+ responses can be largely ascribed to developmental changes in mGlu5 receptor expression (21, 26, 27). For example, Di Giorgi Gerevini et al. (27) determined that mGlu5 was the predominant mGlu subtype expressed embryonically and in early postnatal periods, peaking around P10 before declining to about 50% of that level. Moreover, these authors detected mGlu5 immunostaining on cell nuclei throughout the brain. Taken together, these findings point to an important role for cell surface and nuclear mGlu5 receptors in the control of brain development.

Both Sodium-dependent Transporters and xCT Exchangers Transfer Agonist to Nuclear Receptors—In order to be functional, intracellular receptors must have access to a ligand. Previously, we found that the mGlu5 ligand binding domains were within the lumen of the nuclear envelope exactly as would be predicted from cell biological studies (28). Thus receptor agonists must traverse both the cell surface lipid bilayer as well as the ER membrane which is continuous with the outer nuclear membrane. That this occurs in the striatal system can be shown directly via anti-quisqualate antibodies as well as ligand uptake experiments (Figs. 4–6). Mechanistically, agonist transport across these membranes requires either the sodium-dependent transporter and/or the xCT exchanger. The carrier that predominates appears to be cell type-specific; hence sodium-dependent transporters play a larger role in striatal cultures than in HEK cells.

Neuronal transport also appears to be ligand-specific as quisqualate uptake is largely mediated by the xCT both at the nuclear as well as the plasma membrane. These results are consistent with studies indicating that quisqualate does not serve as a substrate for EAAT1–3 (29). However, it may serve as a substrate for EAAT4. Conceivably, the sodium-dependent component of extracellular quisqualate uptake in dissociated striatal cultures is due to plasma membrane EAAT4 transporters. The lack of EAAT4 on the nuclear membrane may account for the observation that quisqualate uptake into the nuclear lumen is not a sodium-dependent process nor can it be inhibited by TBOA (Fig. 6A). In contrast, sodium-dependent transport plays a more pronounced role in allowing glutamate entry both across the plasma membrane (60–70%; Fig. 5G) as well as the nuclear membrane (30–40%; Fig. 6B). Inasmuch as EAAT1 (GLAST) and EAAT2 (GLT1) are thought to be largely astrocytic (17) and EAAT3 is highly expressed on striatal membranes both at the cell surface and intracellularly (Fig. 5, C and D), it seems reasonable to propose that EAAT3 is the sodium-dependent carrier on intracellular membranes.

Widely expressed in hippocampal, cerebellar, and striatal neurons, EAAT3 is found on cell bodies and dendrites of glu-
tamatergic and GABA-ergic cell types (17). Enriched on postsynaptic elements surrounding areas of glutamate release (30), EAAT3 transporters are perfectly positioned to provide ligand to intracellular receptors while clearing glutamate from the synaptic cleft. Like mGlu5, a significant amount of EAAT3 is found intracellularly; in cultured neurons less than 30% of the total is on the cell surface (31, 32). Although still poorly defined, it is clear that various signaling pathways can rapidly affect the cellular distribution of EAAT3, including redistribution to perinuclear membranes (32). Of note, even conditions that double EAAT3 cell surface expression still leave about 60% of the transporter within the cell (32). Thus the rapid, dynamic changes of EAAT3 on the plasma membrane may contribute to greater ligand delivery to intracellular receptors.

Under physiological conditions, the xCT is thought to transport cystine into cells in exchange for the efflux of intracellular glutamate. l-Cystine is subsequently reduced providing cysteine for cellular utilization (33). Recent studies (34) suggest that the xCT may provide large amounts of nonsynaptic, extracellular glutamate in the brain, potentially leading to excitotoxicity. In contrast, other findings suggest that under normal conditions there may not be sufficient extracellular cystine to drive cystine-glutamate exchange (35). Thus, as an obligate exchanger, in the presence of high concentrations of extracellular glutamate, the xCT will transfer glutamate into the cell. Quisqualate is also a high affinity substrate for xCT and can be transported into hippocampal neurons (23) as well as striatal cells and nuclei. Although EAAT3 appears to play a larger transport role, there may be conditions in which the xCT predominates. Improved resources to probe xCT function may enhance knowledge of this important exchange mechanism.

Transported Versus Nontransported Ligands—Recently, Kniazeff et al. (24) created chimeric mGlu5/GABABR1 receptors (mG5C1) that were unable to be trafficked to the plasma membrane, whereas a mutant, mG5C1ASA, was appropriately distributed. In theory, this might be an ideal model in which to test whether an extracellular ligand can activate an intracellular receptor. In practice, however, the GABABR1 trafficking motif is an RXXR ER retention signal that is part of the ER quality control system. As such it is primarily used to prevent incorrectly folded and/or assembled proteins from getting out of the ER. Two recent papers elegantly make this point. First, by using various extended versions of the RXXR motif in GABABR1, Gassmann et al. (36) showed that ER retention of this motif is probably due to the lack of appropriate binding proteins that would normally position the RXXR sequence into a so-called “operational zone.” These workers proposed that proteins such as COP1 and/or 14-3-3 might mediate this effect in conjunction with GABABR2. Thus, the ER-retained mG5C1 chimera failed to elicit a Ca2+ response because it was held in check by the ER quality control system. In contrast, when the RXXR sequence was mutated to ASAR, it could be trafficked out of the ER to the cell surface or other intracellular compartments such as the nuclear membrane (Fig. 7). In agreement with these results, Goudet et al. (37) have recently shown that in order for the mG5C1 construct to get out of the ER, and

![Fig. 7. Cellular localization and agonist-induced Ca2+ responses of mG5C1 and mG5C1ASA.](image-url)
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hence be functional, it has to interact with an mGlu5/GAB-A/B2 chimera.

Immunocytochemical, pharmacological, and biochemical techniques re-enforce the notion that even without cell surface receptors, glutamate or a transported agonist like quisqualate can affect intracellular Ca\(^{2+}\) levels. As a nonendogenous ligand, quisqualate exemplifies the role that extracellular ligand plays in activating intracellular receptors. Blocking such responses may require different types of antagonists. Thus LY393053 or LY367366 will only block plasma membrane-triggered responses such as those induced by DHGP, whereas MPEP blocks both plasma membrane and intracellular receptor-mediated responses (Fig. 8). The finding that nuclear Ca\(^{2+}\) oscillations are also seen following DHGP treatment, albeit smaller and later than those seen with glutamate or quisqualate (Figs. 8 and 9), presumably reflects the presence of functional receptors on ER membranes. Ca\(^{2+}\)-activated Ca\(^{2+}\) release, and/or Ca\(^{2+}\) entry through nuclear pore complexes. This notion is supported by results showing that direct application of DHGP to isolated mGlu5-expressing nuclei does not trigger nuclear Ca\(^{2+}\) changes. Conversely, cytoplasmic Ca\(^{2+}\) oscillations are seen following quisqualate treatment in the presence of the LY compounds suggesting Ca\(^{2+}\) efflux from the nucleus (Fig. 8). Temporal differences in agonist-mediated Ca\(^{2+}\) responses are consistent with the premise that DHGP and glutamate are accessing different receptor pools leading to responses that can be selectively blocked by a given antagonist (Fig. 8). Taken together, these data suggest that ligands might be tailored for even more selective applications based on the cellular distribution of the receptor.

A proposed model for striatal synapses based on current findings as well as published reports is shown in Fig. 11. As indicated, glutamate released presynaptically can either be taken up by the xCT exchanger or by one of the sodium-dependent transporters. These same proteins provide further passage into luminal domains leading to the activation of resident receptors. Such a model is not without perplexities, however. As described, the xCT is predicted to carry glutamate out of the cell under normal conditions. Moreover, given their predicted topology, sodium-dependent transporters must run in “reverse.” Reverse transport is a widely recognized process whereby alterations in transmembrane gradients, energy deprivation, or substrate heteroexchange lead to transmitter efflux versus uptake (17, 38). In the case of the EAATs as well as the dopamine transporter, selective ions or novel substrate analogs have supported the notion that transporter uptake and efflux are separate, independent processes, although the mechanisms underlying these dissociated modes of operation are as yet unclear (39, 40). Further support for EAAT-mediated intracellular glutamate transport comes from the recent identification of EAAC1 as an important contributor to glutamate transport in cardiac mitochondria. Despite the lack of an inner mitochondrial targeting sequence, EAAC1 transports glutamate across this membrane for use in the malate/aspartate shuttle (41). As is true for this study, protein targeting, topology, and structure/function issues have yet to be addressed for inner mitochondrial transporters. Regardless, the current results suggest that, at least during periods of high activity and/or sustained release, intracellular uptake of glutamate via sodium-dependent or chloride-dependent transporters can lead to activation of intracellular receptors.

One possible caveat to the idea that glutamate activates intracellular receptors is the potentially high levels of glutamate already within the cell. Intracellular glutamate concentrations are difficult to assess, although 10 mM is frequently used as a cytoplasmic value with levels ranging up to 100–200 mM within vesicles (30). In GABA-ergic neurons however, glutamate levels are thought to be much lower because of its role as a GABA precursor. Moreover, anti-glutamate immunogold electron microscopy studies indicate that particles representing glutamate cluster over cytoplasmic organelles such as mitochondria, rough ER, and the nucleus (42). Although these sites may reflect the role of glutamate in metabolism, they may also represent specific transport processes.

**Functional Consequences of Nuclear mGlu5 Receptor Activation**—Little is known about Ca\(^{2+}\) regulation in the nuclei of neurons. As described, Ca\(^{2+}\) can enter the nucleoplasm via the nuclear pore complex or it can be released into the nucleoplasm from the nuclear lumen. Studies dating from a decade ago have shown that the latter site serves as a functional Ca\(^{2+}\) store (43–45). Presumably, Ca\(^{2+}\) release from the lumen of the nuclear membrane would amplify Ca\(^{2+}\) signals arriving via the nuclear pore complex and/or independently generate nucleoplasmic Ca\(^{2+}\) transients. In this study, the presence of nuclear mGlu5 receptors appeared to generate a faster nuclear response in heterologous cells. This model is bolstered by data documenting the presence of IP\(_3\) and ryanodine receptors on inner nuclear membranes (46, 47). These Ca\(^{2+}\)-release channels are perfectly poised to regulate nucleoplasmic Ca\(^{2+}\) levels. Very recent physiological data have confirmed the presence of IP\(_3\) receptors on inner nuclear membranes of Purkinje neurons, although none were present on cerebellar granule nuclei (48).
These data suggest that there may be distinct mechanisms of regulating nuclear Ca\textsuperscript{2+} within different types of neurons. Indeed, the present study extends this concept further by demonstrating unique distributions of nuclear receptors coupled to IP\textsubscript{3} metabolism that may generate such a signal in situ.

What are the functional consequences of nuclear receptor activation? Changes in nuclear Ca\textsuperscript{2+} play an integral role in cellular functions such as protein import, apoptosis, and gene transcription (49). Of note here, varying levels of nuclear Ca\textsuperscript{2+} can activate different transcription factors. For example, the transcription factors NF-\textit{B} and c-Jun N-terminal kinase are selectively activated by large transient Ca\textsuperscript{2+} rises, whereas nuclear factor of activated T cells is activated by low, sustained Ca\textsuperscript{2+} plateaus (50). Thus the amplitude and duration of calcium signals may lead to differential activation of various transcriptional regulators, co-activators, and/or modifying enzymes (51). One prominent nuclear target may be calcium/calmodulin-activated kinase IV. This kinase is known to phosphorylate CREB within the nucleus (52). Very recently this kinase and/or its other nuclear isoforms have been implicated in long term memory consolidation. Specifically, Limback-Stokin et al. (53) demonstrated in vivo that nuclear calcium signaling pathways, not cytoplasmic, were responsible for converting short term memory into long term memory. Conceivably, mGlu5-mediated changes in nuclear Ca\textsuperscript{2+} play a critical role in this fundamental property as well.

Although rises in nucleoplasmic Ca\textsuperscript{2+} may affect transcription, recent studies have shown that depletion of luminal Ca\textsuperscript{2+} displaces a mass, possibly representing cargo, within the nuclear pore complex itself. Luminal Ca\textsuperscript{2+} loss is mediated by the activation of the IP\textsubscript{3} or the ryanodine receptor, because specific inhibitors of these calcium channels block changes in the conformation of the complex (54). Thus another potential consequence of the activation of nuclear mGlu5 may be the indirect modulation of nuclear pore complexes. Taken together these data suggest that alterations in the amplitude, frequency, and duration of nuclear Ca\textsuperscript{2+} may have profound effects upon many processes, including transport and transcription.

These data help establish the role of nuclear mGlu5 receptors in a native, physiological setting. Moreover, contrary to the idea that intracellular mGlu5 receptors are nonfunctional and merely constitute an internal reserve of receptors waiting to go...
to the cell surface, the present data argue strongly for a
dynamic intracellular role in signal transduction. Given that the
ER and nuclear lumen serve as unique intracellular stores of
Ca\textsuperscript{2+}, intracellular receptors such as mGlu5 may play a pivotal
role in generating and shaping intracellular Ca\textsuperscript{2+} signals.

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