Direct interactions between NMDA and D_1 receptors: a tale of tails

F.J.S. Lee* and F. Liu*†‡§[1]

*Department of Neuroscience, Centre for Addiction and Mental Health, Clarke Division, University of Toronto, Toronto, ON, Canada M5T 1R8, †Department of Physiology, University of Toronto, Toronto, ON, Canada M5T 1R8, ‡Department of Psychiatry, University of Toronto, Toronto, ON, Canada M5T 1R8, and §Institute of Medical Science, University of Toronto, Toronto, ON, Canada M5T 1R8

Abstract

Considerable evidence has accumulated describing a complex interaction between the dopaminergic and glutamatergic pathways. Efforts to describe the mechanisms underlying this complex interaction have implicated a functional interaction between dopamine and glutamate receptors. Classically, the interaction between D_1 and NMDA (*N*-methyl-D-aspartate) receptors has been proposed to involve the activation of second-messenger signalling cascades after receptor stimulation. However, in recent years, another paradigm has emerged which involves the direct interaction between D_1 and NMDA receptors. The physical association between D_1 and NMDA receptors is unique in that two different regions of the D_1 C-terminus are able to couple specifically and physically with two different NMDA subunits. The selective modulation of multiple NMDA receptor-mediated functions by direct interactions with D_1 receptors may form a new avenue to identify specific targets for therapeutics to modulate NMDA receptor-governed synaptic plasticity, neuronal development and disease states.

Introduction

The overlap and convergence of both dopaminergic and glutamatergic projections in the mammalian brain provides the architectural framework for complex neuronal interactions. Underlying the complexity of dopamine–glutamate interactions is the co-localization of dopamine and glutamate receptors, including D_1 and NMDA (*N*-methyl-D-aspartate) receptors, within several brain structures including caudate-putamen, nucleus accumbens, hippocampus and rat forebrain as determined through autoradiography and immunocytochemistry [1–11]. Dopamine-glutamate cross-talk and feedback pathways may in part be attributed to functional interactions between these two receptors. Dopamine D_1 and NMDA receptors represent two functionally and structurally diverse receptor classes. The D_1 receptor is a member of the large superfamily of G-protein-coupled receptors that typically possess seven transmembrane domains, which activate signal-transduction pathways through G-proteins. NMDA receptors are ionotropic receptors that are ligand-gated ion channels and are composed of multiple subunits. NMDA receptors, with their ability to conduct ion fluxes, facilitate fast excitatory synaptic transmission.

Previous studies have shown that the functional interaction between D_1 and NMDA receptors can be attributed to the cross-talk in signal-transduction cascades activated by the respective receptors. D_1 receptor stimulation that leads to downstream activation of protein kinase A has been shown to be involved in the phosphorylation of the NR1 subunit of the NMDA receptor [12] and NMDA-dependent long-term potentiation [13]. Activation of the D_1 receptor has also been shown to modulate excitatory post-synaptic currents [14] and affect NMDA currents through protein kinase C-dependent mechanisms [15,16]. Furthermore, modulation of both NMDA currents and NR1 phosphorylation appears to involve the DARPP-32 [12,16]. Receptor trafficking also appears to be affected by the cross-talk between these two receptors. D_1 receptor activation has been shown to affect NMDA receptor subcellular localization [17,18], whereas D_1 receptor cell-surface localization appears to be up-regulated after NMDA activation [19,20]. These examples show the diversity and complexity of the D_1–NMDA receptor interaction. However, it has been recently shown that GABA (γ-aminobutyric acid)-A receptors can directly interact with D_5 receptors and that this interaction affects both GABA-A and D_5 receptor function [21]. Similar to NMDA receptors, GABA-A receptors are ligand-gated channels that mediate fast inhibitory synaptic transmission. The D_5 receptor CT (C-terminus) was able to interact directly with GABA-A receptor CT. In the presence of D_5 receptor agonist, GABA-A receptor activity was significantly reduced. In contrast, GABA-A receptor activation significantly inhibited D_5-receptor-mediated cAMP accumulation. Given these results, we speculated on the possibility that D_1 receptors are also capable of directly interacting with NMDA receptors. These results were reported in two recently published papers [20,22].

Key words: brain, D_1 receptor, dopamine, glutamate, *N*-methyl-D-aspartate receptor (NMDA receptor).

Abbreviations used: CaM, calmodulin; CT, C-terminus; GABA, γ-aminobutyric acid; GST, glutathione S-transferase; NMDA, *N*-methyl-D-aspartate; PI3K, phosphoinositide 3-kinase.

[1]**To whom correspondence should be addressed (email f.liu.a@utoronto.ca).**

Biochemical evidence for D_1–NMDA receptor interaction

To provide evidence for the presence of a D_1–NMDA receptor complex, we have shown through a series of co-immunoprecipitation experiments from both rat primary neuronal cultures (hippocampal and striatal) and COS-7 cells co-expressing the D_1 and NMDA receptor that these two receptors indeed interact [20,22]. Furthermore, affinity precipitation/'pull-down' assays using GST (glutathione S-transferase) fusion protein constructs encoding the CT tails of the D_1 receptor or NMDA receptor subunits NR1-1a and NR2A, confirmed that the D_1–NMDA receptor interaction is mediated by the CT of these two receptors. However, these results did not clarify if the interaction between D_1 and NMDA receptors is mediated through an accessory protein or through direct protein–protein interactions. Therefore to examine the nature of the interaction, we employed a series of *in vitro* binding and blot overlay assays to examine the possibility that the D_1–NMDA interaction is mediated by direct physical interaction between the CT of both these proteins. Not only did these experiments confirm the interaction between D_1 and NMDA receptors initially detected by co-immunoprecipitation studies but they also strongly suggested that the interaction may be a result of direct protein–protein interactions between these two receptors. Surprisingly, the CT of both the NR1-1a and NR2A subunits was able to interact directly with the D_1 receptor. Therefore we speculated that different domains in the D_1 CT were mediating the specific interaction with either the NR1-1a or NR2A subunit. To delineate these specific motifs, GST fusion protein constructs were created that encoded smaller regions of the D_1 CT: D_1-t1 (Ala^{357}-Asn^{386}), D_1-t2 (Leu^{387}-Leu^{416}) and D_1-t3 (Ser^{417}-Thr^{446}). Through blot overlay assays and confirmed with *in vitro* binding assays, not only were we able to identify more discrete regions of the D_1 CT tail mediating these direct protein interactions, we were also able to show that the D_1-t2 peptide interacts with NR1-1a subunit, whereas D_1-t3 interacts with NR2A subunit. Interestingly, D_1 receptor activation resulted in a decrease in the D_1–NR1 interaction but did not affect the D_1–NR2A interaction. This suggests that D_1 receptor stimulation may play a key role in defining the strength of the two interactions mediating the D_1–NMDA receptor coupling.

D_1–NMDA receptor interaction modulates NMDA currents

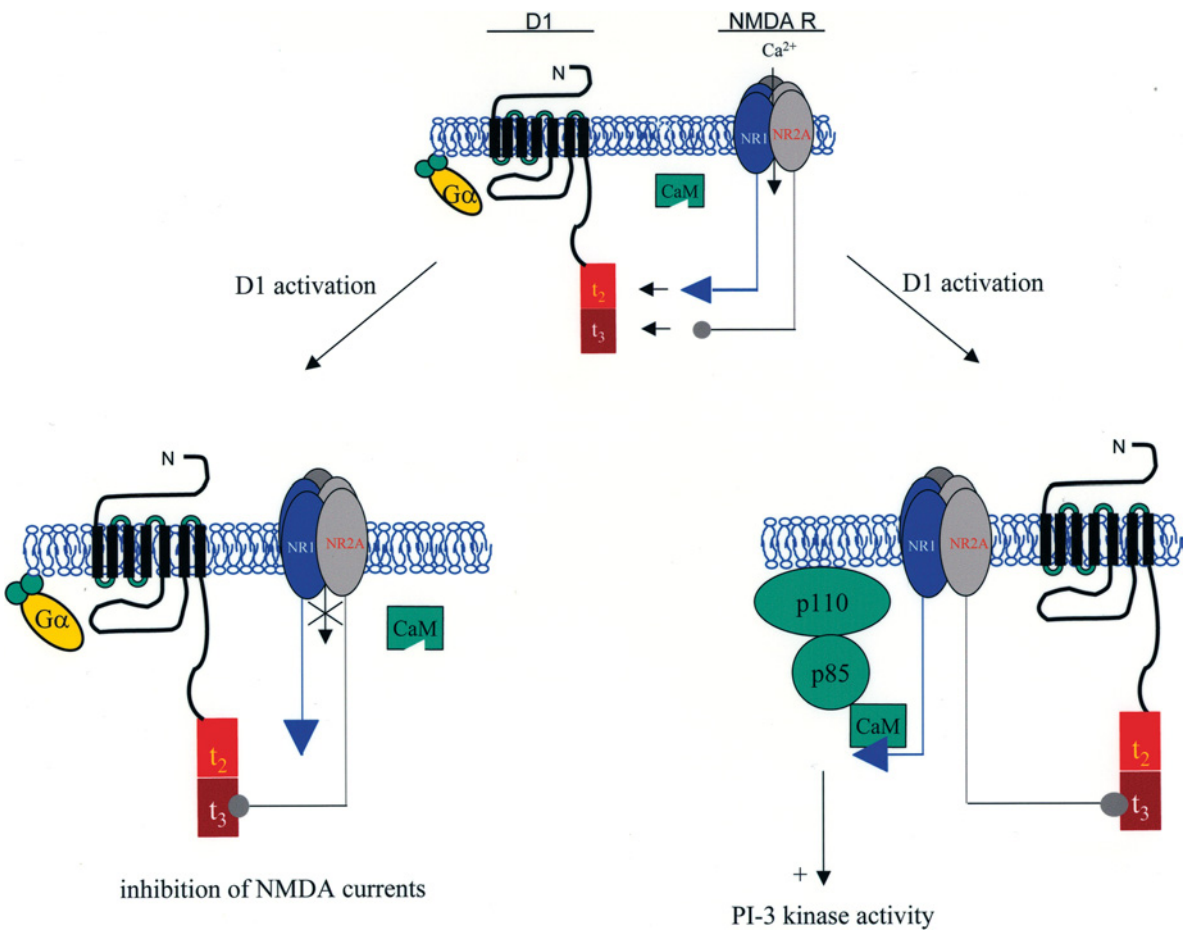
Although we were able to identify biochemically two discrete protein–protein interactions which facilitate the physical coupling of the D_1 receptor with the NMDA receptor, the functional consequences of these interactions remained unclear. One obvious possibility is that NMDA currents may be affected by the interaction with D_1 receptors. To address this possibility, we measured whole cell currents from both dissociated primary rat hippocampal neuronal cultures and from HEK-293 (human embryonic kidney 293) cells co-expressing D_1 receptors and NR1-1a/NR2A subunits. Electrophysiological recordings revealed that NMDA currents were significantly reduced after D_1 receptor activation by agonist SKF81297. Furthermore, this reduction in NMDA currents mediated by the D_1 receptor agonist was almost completely abolished by the application of the D_1-t3 peptide but not by D_1-t2. Interestingly, through the use of a cell-based colorimetric assay, the reduction in NMDA current after D_1 receptor activation was paralleled by a similar decrease in NMDA receptors at the cell surface. Similar to the electrophysiology experiments, the reduction in NMDA receptors on the cell surface was blocked by the D_1-t3 peptide only. In addition, these studies were performed in the presence of protein kinase A and C inhibitors, which suggest that these effects on both NMDA currents and receptor subcellular localization can be attributed to the physical interaction between D_1 and NMDA receptors. Other studies have reported an increase in NMDA receptor mobilization and/or NMDA currents after D_1 receptor stimulation [15–18]. These seemingly discrepant effects may in part be explained by differences in NMDA receptor subunit composition and by the fact that D_1 receptor activation can still modulate NMDA receptor activity indirectly through second-messenger signalling cascades. However, in our study, the inability of a non-hydrolysable analogue of GTP that prevents receptor-mediated activation of G-proteins, GTP[S], to reverse the inhibitory effects of D_1 activation on NMDA currents, in addition to the use of protein kinase inhibitors, suggests that the observed functional outcome of the D_1-t3–NR2A interaction is specifically due to the direct protein–protein interaction between these two proteins. Taken together, it not only appears that the D_1/NMDA receptor direct interaction facilitates the D_1 receptor activation-mediated reduction in NMDA currents but that it does so by inducing a decrease in cell-surface localization of NMDA receptors (Figure 1).

D_1–NMDA receptor interaction affects NMDA-mediated excitotoxicity

Another prominent functional outcome of NMDA receptor stimulation, especially when unchecked, is excitotoxicity. Overactivation of NMDA receptors can potentially lead to Ca^{2+} overload that in turn could have detrimental effects on the mitochondria that lead to cell death. Given the D_1 receptor-mediated inhibition of NMDA currents, we speculated that the D_1–NMDA interaction could also potentially attenuate NMDA receptor-mediated excitotoxicity. As predicted, in both rat hippocampal neuronal cultures and HEK-293 cells co-expressing D_1 and NMDA receptors, pretreatment with the D_1 receptor agonist SKF81297 mitigated the excitotoxicity induced by excessive NMDA receptor stimulation as indexed by a 40–50% reduction in apoptotic cells. However, we were surprised to discover that this effect was abolished not by co-expression of D_1-t3, as seen earlier with the effects on NMDA currents, but by D_1-t2. Therefore the D_1 receptor-mediated protective effects appear to be independent of the effects on NMDA currents.

Figure 1 | A model of the direct interactions between D_1 and NMDA receptors

Activation of D_1 receptors results in the dissociation of the D_1-t2 region of the D_1 receptor from the NR1 CT. This allows the recruitment of CaM and PI3K to the NR1 CT and leads to the activation of the PI3K. Activation of PI3K activity leads to cell survival. Agonist activation of D_1 receptor also leads to the inhibition of NMDA currents through the D_1-t3–NR2A interaction which results in a decrease in receptors localized at the cell surface.



To provide insight into potential alternative pathways and mechanisms involved, we found that preincubation with the PI3K (phosphoinositide 3-kinase) inhibitor wortmannin completely abolished the D_1 receptor-mediated modulation of NMDA-induced apoptosis. In addition, utilizing fibroblast lines from PI3K p85α-null embryos [23] co-transfected with D_1 and NMDA receptors, D_1 receptor activation exhibited no protective effects on NMDA-induced apoptosis thereby implicating PI3K as a critical component for the D_1 receptor-mediated protection from NMDA-induced neurotoxicity. Furthermore, we have evidence that these protective effects may be due to an increased association of the NR1 subunit with CaM (calmodulin) after D_1 receptor activation. Previous studies have shown that CaM, which directly binds to PI3K [24,25] and can also interact with NR1 subunit [26,27], has an important role in the activation and accumulation of PI3K. D_1 receptor activation not only resulted in a decrease in D_1–NR1 complex but it also led to an increase in the association of the NR1 subunit with CaM, as determined through co-immunoprecipitation assays. In addition, the D_1 receptor agonist SKF81297 increased the association of PI3K with NR1 subunits and increased PI3K activity itself, an effect that was blocked by CaM antagonist W-13 or through co-expression of the D_1-t2 mini-gene.

These results surprisingly suggest that the D_1–NMDA interaction is not only mediated by two different sites of interaction but that each of these sites are functionally distinct: the D_1-t3–NR2A interaction affects NMDA receptor currents, whereas the D_1-t2–NR1-1a interaction appears to involve CaM–PI3K signalling complex.

The D_1–NMDA interaction also affects D_1 receptor functions

In addition to the D_1–NMDA receptor complex having effects on NMDA receptor functions, we have also demonstrated that there are functional effects on D_1 receptor-mediated signalling events. In COS-7 cells co-expressing D_1

and NMDA receptors, pretreatment with NMDA increased D_1 receptor-mediated cAMP accumulation by approx. 45%. Experiments with the D_5 receptor did not exhibit similar effects. This effect was not only D_1-receptor-specific, but it could also be blocked with the D_1 antagonist SCH23390 and the NMDA receptor antagonists AP-5 and MK-801. Furthermore, similar to the effects on excitotoxicity, the increase in cAMP accumulation is dependent on the D_1-t2–NR1-1a interaction mediated by the CT of both proteins. Given that receptor trafficking is a mechanism to modulate receptor function, we employed both immunocytochemistry and cell-based colorimetric assays to determine if there is a change in D_1 receptor localization after NMDA receptor activation. Interestingly, we were able to show that after NMDA treatment there appears to be a mobilization of D_1 receptors to the cell surface, which could account for the increase in D_1 receptor-mediated cAMP accumulation. Furthermore, it appears that the increase in D_1 receptors to the cell surface is a result of increased insertion of new receptors to the plasma membrane, as opposed to a decrease in the internalization of cell-surface receptors. Pretreatment of cells with tetanus toxin, which selectively cleaves vesicle-associated membrane protein and prevents exocytosis, effectively abolished the enhancement of D_1 receptor localization at the cell surface. In addition, tetanus toxin treatment also effectively blocked the increased cAMP accumulation after NMDA receptor activation.

The final tail: D_1-t2/NR-C1

Although we had delineated discrete regions within the D_1 receptor CT that mediate the D_1–NMDA receptor interaction, it was still unclear as to which specific region within the CT of the NR1-1a subunit was essential for the direct coupling with D_1 receptors. Again we constructed GST fusion proteins that encoded smaller domains of the NR1-1a CT: NR-C0 (Glu^{834}-Asp^{864}), NR-C1 (Asp^{864}-Thr^{900}) and NR-C2 (Thr^{900}-Ser^{938}). Using a series of affinity purification experiments and *in vitro*-binding assays, we were able to show that the NR-C1 domain appears to be essential for the D_1-t2–NR1-1a interaction. This was confirmed in cAMP assays in which the increase in D_1 receptor-dependent cAMP accumulation after NMDA receptor activation was inhibited after co-expression of a NR-C1 mini-gene. Furthermore, the increase in D_1 receptor localization after NMDA treatment was also abolished with co-expression of the NR-C1 mini-gene. In both instances, neither the NR-C0 nor NR-C3 had any effect.

Summary

The direct interaction between D_1 and NMDA receptors and the previous discovery of a D_5–GABA-A receptor complex may provide a novel mechanism by which these receptors can quickly modulate each other. Additionally, the direct interaction may also provide a functional complex in which both proteins are accessible to other proteins. For example, the functional interaction between D_1-t2 and NR1-1a has direct implications on the interaction between NR1-1a with CaM.

Although we have described two different sites on the D_1 receptor CT that interact with different NMDA subunits, it remains unclear as to whether both discrete interactions can occur between one individual D_1 receptor and one individual NMDA receptor or whether, within a population of D_1 and NMDA receptors, there are groups of receptors that interact through the D_1-t2–NR1-1a interaction and another group that interact through the D_1-t3/NR2A. Furthermore, the D_1–NMDA interaction does not exclusively affect NMDA receptor activity. D_1 receptor function is also affected by NMDA receptor activation. It appears that while NMDA activity may recruit D_1 receptors to the cell surface, D_1 activation appears to result in the internalization of NMDA receptors from the cell surface. Given the diversity of NMDA receptor subunit composition and the vast array of proteins that interact with both these receptors, these two direct protein–protein interactions provide a mechanism by which functional modulation of both receptors can be finely tuned. It will be interesting to identify which mechanism is more affected in disease states such as schizophrenia and stroke.

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