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**COMMENTARY**

# Src, *N*-Methyl-D-aspartate (NMDA) Receptors, and Synaptic Plasticity

*Michael W. Salter*

Division of Neuroscience, Hospital for Sick Children, Toronto, Ontario; and Department of Physiology, University of Toronto, Toronto, Ontario, Canada

**ABSTRACT.** The protein tyrosine kinase Src is expressed widely in the central nervous system and is abundant in neurons. Over the past several years, evidence has accumulated showing that one function of Src is to regulate the activity of *N*-methyl-D-aspartate (NMDA) receptors and other ion channels. NMDA receptors are a principal subtype of glutamate receptor that mediates fast excitatory transmission at most central synapses. Recently it has been discovered that, by means of up-regulating the function of NMDA receptors, Src mediates the induction of long-term potentiation (LTP) in the CA1 region of the hippocampus. This finding led to a new model for induction of LTP whereby tetanic stimulation produces a rapid activation of Src, causing enhanced NMDA receptor function. This enhanced NMDA receptor function boosts the entry of $Ca^{2+}$, which may thereby trigger the downstream signalling cascade, ending in potentiation of non-NMDA receptors. This functional role for Src may be important in physiological and pathophysiological processes in the central nervous system. BIOCHEM PHARMACOL **56**;7:789–798, 1998. © 1998 Elsevier Science Inc.

**KEY WORDS.** tyrosine kinase; long-term potentiation; phosphorylation; hippocampus; NMDA receptor; spinal cord dorsal horn

In the CNS, there is a diversity of PTKs†, many of which are known to be important for growth, proliferation, and differentiation [1]. Consequently, it had been generally thought that the role of PTKs is in the ontogeny of the CNS. It might thus appear paradoxical that in the mature CNS there are high levels of PTKs [2], and that many are expressed in neurons, which are post-mitotic cells. Over the past several years, it has become apparent that one function of PTKs is to regulate the activity of ion channels in neurons [3–7]. One type of ion channel found to be regulated by PTKs is the NMDA receptor, a main class of glutamate receptor in the CNS [3]. An endogenous PTK that has been identified as regulating NMDA receptor function is Src, the non-receptor PTK [8]. Src is one of the most well-known and most extensively studied of the tyrosine kinases, but, paradoxically, its functions in the CNS have been enigmatic. Recent observations indicate that the regulation of NMDA receptors by Src may mediate the induction of a form of synaptic plasticity, known as LTP, in the hippocampus. Below is outlined the evidence for Src regulation of NMDA receptors, the role of this function in LTP induction, and some of the challenges that lie ahead.

* Correspondence: Dr. Michael W. Salter, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario M5G 1X8, Canada. Tel. (416) 813-6272; FAX (416) 813-7921; E-mail: mike.salter@utoronto.ca

† *Abbreviations:* LTP, long-term potentiation; mEPSC, miniature excitatory post-synaptic currents; NMDA, *N*-methyl-D-aspartate; NR, NMDA receptor; PTK, protein tyrosine kinase; SH2, Src-homology 2; and SH3, Scr-homology 3.

## SRC IN THE CNS

PTKs fall into two main groups: receptor and non-receptor PTKs [9, 10]. Receptor PTKs have a single transmembrane and intracellular region that often contains more than one copy of the catalytic domain. These PTKs are activated by the binding of signalling molecules, such as growth factors [11, 12], to the receptor site in the extracellular domain. In contrast, non-receptor or cytoplasmic PTKs are not directly activated by extracellular ligands. Non-receptor PTKs are intracellular proteins with one catalytic domain, generally located near the C-terminus. They are typified by the protooncogene gene families *src*, *abl*, and *fps* [1, 13].

Src is the prototype of the group of non-receptor PTKs [14], and it has well-known structural features, as represented in Fig. 1. Two regions of Src, the SH2 and SH3 domains, have received much attention, as similar domains are found in numerous proteins, and interactions via these domains are widely involved in cell signalling [15, 16]. The SH2 and SH3 domains not only mediate protein–protein interactions but also bind intramolecularly, resulting in folded conformations of the protein in which the enzymatic activity is suppressed [14, 17, 18].

While the main isoform of Src expressed by all cells is c-Src, it was discovered many years ago that neurons express a high-activity isoform of Src called n-Src [19, 20]. n-Src was found to contain an alternatively, spliced cassette of six amino acids inserted after amino acid 114, i.e. in the SH3 domain, of c-Src [20]. More recently, it has been found that another distinct cassette may be inserted alone or in

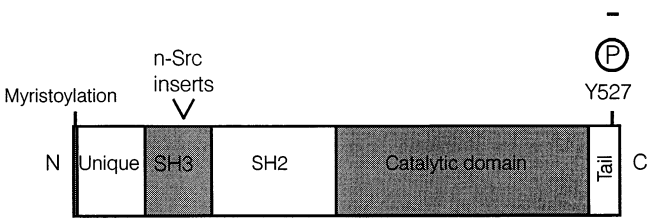


**FIG. 1. Features of the domain structure of Src. Also shown are the N-terminal site for myristoylation, which is necessary for membrane localization, and the C-terminal negative regulatory site.**

tandem, generating a number of neuronal isoforms of Src [21].

Src is a member of a family of tyrosine kinases having a total of nine members, five of which—Src, Fyn, Lyn, Lck, and Yes—are known to be expressed in the CNS. All members of the Src family of non-receptor PTKs show the general domain structure described above [14]. The catalytic, SH2, SH3, and C-terminal domains are highly homologous in the different Src family kinases. Where the various members differ most is in the so-called unique domain, a region of low sequence conservation near the N-terminus. It has been suggested that the unique domain may simply be a spacer in the molecule [22]. However, unique domains in members of the Src family have been found to be crucial for directing the kinase and permitting the phosphorylation of particular substrates [23, 24]. Thus, even though it is outside the catalytic region, the unique domain may regulate the function of Src kinases.

Src is expressed widely in the CNS with high levels of expression in the cerebral cortex, hippocampus, pons, midbrain, cerebellum, and spinal cord [25, 26]. Within CNS neurons, Src is found both pre- and post-synaptically. The post-synaptic localization is particularly relevant with respect to the modulation of NMDA receptor function, as Src has been found in the post-synaptic density (PSD) [27, 28], the main structural component of excitatory synapses where glutamate receptors are concentrated.

## REGULATION OF NMDA RECEPTORS BY SRC

The NMDA receptor, as a main subtype of glutamate receptor, participates in rapid excitatory synaptic transmission throughout the CNS [29]. NMDA receptors are members of the superfamily of ligand-gated ion channels, and molecular cloning has led to the identification of a variety of NMDA receptor subunit proteins (NR1, NR2A–D, NR-L/X1) [30, 31]. Native NMDA receptors appear to be heteroligomeric complexes with the M2 region of the subunits coming together as a central pore selectively permeable to cations [30, 32]. The receptors likely consist of one or more NR1 subunits together with NR2A–D subunits [33] and possibly the more recently identified subunit NR-L/X1 [34, 35].

NMDA receptor activation requires glycine, a co-agonist that binds to an extracellular site on the channel complex [36]. The activated channels are permeable to monovalent cations, such as $Na^+$ and $K^+$, and also to divalent cations, the most important of which is $Ca^{2+}$ [37, 38]. A key process regulating NMDA receptor function is phosphorylation [39], although NMDA receptors are known to be regulated [29] at diverse extracellular [40–44] as well as intracellular [45] sites. Both serine/threonine [46–55] and tyrosine [3] phosphorylation have been shown to regulate NMDA receptor function.

In terms of NMDA receptor regulation by PTKs, it was found that currents through native NMDA receptors are increased by tyrosine phosphorylation [3], and this has also been observed with recombinant NMDA receptors [56, 57]. Previously, for muscle nicotinic receptors it had been found that tyrosine phosphorylation increases the rate of desensitization [58]. Thus, the effect of PTKs on NMDA receptor function is quite different from that on the function of nicotinic receptors. Like that of NMDA receptors, the function of γ-aminobutyric acid type-A ($GABA_A$) receptors has also been found to be increased by tyrosine phosphorylation [6, 59]. Hence, it may be that tyrosine phosphorylation is a general post-translational mechanism for regulating the function of ligand-gated ion channels in the CNS.

Recently, we set out to identify the endogenous PTK regulating NMDA receptor function [8]. The first step in our strategy was to determine whether the PTK was a member of the Src family of non-receptor kinases. For this purpose we used a peptide, EPQ(pY)EEIPIA [60], that has high affinity for the SH2 domain of kinases in the Src family. As mentioned above, the SH2 domain is involved in negatively regulating Src kinase activity, which occurs through intramolecular binding of the phosphorylated tyrosine in the tail of the kinase. The EPQ(pY)EEIPIA peptide competes for this intramolecular binding and thereby precludes the auto-inhibition of Src family kinases [60]. Applying this peptide to the cytoplasmic face of inside-out patches was found to increase NMDA channel activity, whereas the non-phosphorylated form of the peptide, EPQYEEIPIA, which does not affect kinase activity, had no effect on NMDA channels. In complementary experiments, we used anti-cst1 [61], an antibody that inhibits Src family kinases [62], and found that it decreases NMDA channel activity, while control IgG had no effect. These results indicated that the endogenous PTK regulating NMDA receptor function is a member of the Src family.

Src was identified as the specific member regulating NMDA channel function by testing an antibody, anti-src1, that was known to block selectively the function of Src but not that of other members of the Src family [62]. Anti-src1 caused a decrease in basal NMDA channel activity. Moreover, anti-src1 prevented the effect of EPQ(pY)EEIPIA, indicating that Src is necessary for the effect of the activating peptide. In other experiments, applying exogenous recombinant $pp60^{c\text{-}src}$ increased NMDA channel activity, but this was prevented by heat-inactivating the kinase just prior to use.

As anti-src1 does not bind to the catalytic domain of Src, we envisaged two potential mechanisms by which it may act. The first is that the binding of the antibody, which is relatively large in size in comparison with Src, might sterically hinder enzymatic activity. Alternatively, it is possible that anti-srcl acts by blocking an interaction between the region in Src that the antibody recognizes, amino acids 40–58, and a complementary region in a target protein. To differentiate between these possibilities, we tested the peptide fragment Src(40–58). This peptide reduced NMDA channel activity when applied to the cytoplasmic face of the membrane. In contrast, a peptide with the same amino acid composition but in random order, "scrambled Src(40–58)," had no effect. Furthermore, pre-administering Src(40–58) prevented the effects of EPQ-(pY)EEIPIA. In other experiments, Src(40–58) was found not to block *in vitro* phosphorylation of a small substrate peptide by recombinant Src, indicating that Src(40–58) does not directly affect kinase catalytic activity. Thus, we concluded that the region 40–58 may interact with a component, most likely a protein, of the receptor complex and that this interaction is necessary for the effect of Src on NMDA channels. The region 40–58 is contained within the unique domain and, therefore, the results indicate that this domain is of functional importance in Src.

Whether or not Src is closely associated with NMDA channels could not be determined from the previous experiments because, while membrane patches are small in comparison with cells, the patches are quite large in comparison with the size of the proteins involved. Thus, even within the membrane patches it is possible that Src was remote from the NMDA receptor complex. However, we found that Src and NMDA receptor subunit proteins co-precipitate one another, demonstrating that Src is associated with the NMDA channel complex. This co-precipitation might be via a direct interaction between Src and an NMDA receptor subunit protein, but alternatively it is possible that Src associates with NMDA channels by means of an intervening adaptor protein.

From kinetic analysis of the NMDA channel activity in the patches, it was concluded that Src increases the channel gating in single activations of the receptors. Because synaptic NMDA responses are due to single activation of the receptors [63], if the synaptically stimulated receptors are affected similarly to the extrasynaptic receptors in the patches, we predicted that the synaptic NMDA responses should be increased by Src. This was confirmed in studies of mEPSCs. Moreover, it was discovered that Src differentially regulates synaptically activated NMDA, but not non-NMDA channels. The mEPSCs represent the response to single quantal release events, and, as such, the synaptically activated NMDA and non-NMDA receptors are almost certainly located apposed to the same presynaptic terminal. Thus, taking all of the information together, we suggest that it is Src that is physically associated with and up-regulates the function of NMDA receptors.

The two principal unresolved questions concern (1) the molecular mechanism, and (2) the functional significance of the regulation of NMDA channels by Src. The question of the molecular mechanism has largely been unexplored, and in the next section the central issues remaining to be examined are outlined. The other principal question—the functional significance of the regulation—has been examined recently, and work related to this is described in the last section.

## WHAT IS THE MOLECULAR MECHANISM FOR THE SRC-INDUCED INCREASE IN NMDA CHANNEL GATING?

This question subsumes two related but distinct issues. The first issue is determining which protein is the molecular target of the phosphorylation, and the second is understanding how it is that phosphorylation of a particular tyrosine residue(s) in that protein is transduced into the alteration in NMDA channel function. Before considering the potential molecular target, we note that it is conceivable that either intracellular or extracellular tyrosine residues might be subject to phosphorylation. However, reagents used in the single-channel studies are membrane impermeant, and these were applied to the cell cytoplasmic face of the patches, which indicates that regulation of the channels is due to phosphorylation at an intracellular site. From this, the simplest explanation for regulation of channel function is that it is produced by phosphorylation in one of the cytoplasmic domains of an NMDA channel subunit protein. It has been reported that NR2A [64], NR2B [65], and NR2D [66] are phosphorylated on tyrosine; there is no evidence that NR1 is tyrosine phosphorylated.

If the target for tyrosine phosphorylation is one of the known NMDA receptor subunits, then the apparent topology and amino acid sequences predicted for the cloned receptors indicate that there are many possible tyrosine residues. One potential intracellular domain is the C terminus, a region which, despite past controversy about the transmembrane topology of glutamate receptors [67, 68], is undoubtedly located intracellularly in NR1 and NR2 subunit proteins [50]. In NR1, the C-terminal region of all of the splice variants contains one tyrosine but, as mentioned above, there is no evidence that NR1 is phosphorylated on tyrosine. The C-terminus of all members of the NR2 group is much longer than the corresponding region in NR1. NR2A contains 25 tyrosines in the C-terminus; there are 24 in NR2B and 26 in NR2D. Another intracellular domain is the short region between the first and second membrane segments. This region contains one tyrosine residue in all NR2 subunits, whereas the other predicted intracellular domain contains no tyrosine residues in any NR2 subunit. Thus, it is clear that there are many potential sites for tyrosine phosphorylation even if we consider only those NMDA receptor subunits that are already known to be phosphorylated on tyrosine.

In studying recombinant NMDA receptors, Köhr and

Seeburg [56] found that whole-cell currents mediated by NR1/NR2A receptors expressed in HEK293 cells were potentiated by Src, whereas those mediated by NR1/NR2B receptors were unaffected. Moreover, they found that Src had no effect on mutant NR1/NR2A receptors in which the C-terminal intracellular tail of NR2A had been deleted. A simple explanation for these findings could be that the target of functionally relevant tyrosine phosphorylation is NR2A and that the site of phosphorylation is a tyrosine, or tyrosines, within the tail of the protein. An alternative, and nearly as simple, explanation may be that the C-terminal tail of NR2A acts as an adaptor allowing Src to bind to the channel complex, where it then phosphorylates a protein that is not NR2A.

The function of NR1/NR2B diheteromeric channels appears to be unaffected by tyrosine phosphorylation, and yet NR2B extracted from rat brain is phosphorylated on tyrosine (see above) and NR2B can be phosphorylated *in vitro* by the Src family kinase Fyn [69]. One potential explanation for this apparent paradox may be that the lack of effect of Src on NR1/NR2B receptors is due to an inability of Src to phosphorylate the NR2B protein receptors in cells in which the receptors are expressed. This might occur, for example, because of lack of an appropriate adaptor protein that would allow Src to bind to the channel complex. An alternative explanation, which has yet to be examined, is that in recombinant NR1/NR2B receptors the NR2B subunit is already fully tyrosine phosphorylated by basal kinase activity. If this is the case, then there might be no non-phosphorylated substrate available for exogenously added Src, and thus its effects may have been occluded. An additional possibility is that there is no paradox and that phosphorylation of NR2B does not affect channel gating but rather subserves another function such as receptor trafficking or targetting.

The situation appears to be even more complex as there may be effects of tyrosine phosphorylation of channels containing NR2B in native receptors. Stocca and Vicini have found that in neurons in the cerebral cortex Fyn potentiates NMDA currents, which are blocked by CP101,606 [70]. NMDA current blockade by CP101,606 is considered a hallmark of receptors containing NR2B, but not NR2A [71]. Given that native receptors often contain two or more types of NR2 subunit, these findings might be explained by phosphorylation of a non-NR2B subunit even in receptors that contain NR2B. But given that NR2B is phosphorylated on tyrosine it seems equally likely that in the native receptors there may be a functional effect of this phosphorylation and that this effect is absent from NR1/NR2B recombinant receptors for the reasons outlined above.

Other work indicates that NMDA receptors lacking the NR2A subunit may be potentiated by Src. Chen and Leonard [57] have reported that Src potentiates NR1/NR2D currents in oocytes. We also have found that either high or low conductance native receptors are potentiated by Src (Yu XM and Salter MW, manuscript in preparation). The relevance of this is that Cull-Candy and colleagues [72] have suggested that low conductance channels do not contain NR2A. Thus, whether phosphorylation of NR2A is necessary for the increase in function by Src of NMDA receptors remains to be established.

A concept that has blossomed over the past few years is that ligand-gated ion channels, and in particular glutamate receptors, are not isolated in the membrane but rather that the receptors are, in fact, multiprotein complexes comprised of receptor subunits *per se* and of associated proteins. For example, non-NMDA receptors have been found to be associated with the proteins AKAP [73] and GRIP [74], and NMDA receptors have been reported to be associated with the proteins PSD-95 [75], SAP102 [76], α-actinin [77], and calmodulin [78]. The observation that there are a number of proteins associated with channels opens up the possibility that the target of the phosphorylation that produces the increase in NMDA receptor function may be one of the associated proteins rather than one of the receptor subunits. The phosphorylated associated protein might then modulate the function of the channel.

What will the ultimate proof of the molecular target be? Superficially, the answer to this seems straightforward—identifying the protein phosphorylated by endogenous Src, determining that mutagenesis of a particular tyrosine or tyrosines in the target protein eliminates the functional effect of endogenous Src, and showing by direct biochemical means that endogenous Src phosphorylates this residue. This is easy enough to say, but there are significant technical hurdles, not the least of which may be the need to fully reconstitute NMDA receptors and all associated proteins in a heterologous expression system. Another potentially confounding factor relates to observations that many proteins are known to be tyrosine phosphorylated at multiple residues. Deducing the stoichiometry of phosphorylation required for the functional changes has not been attempted in the electrophysiological studies, and for native channels it is not clear that determining the stoichiometry would be possible, strictly by using this means. Thus, whether one or more than one tyrosine needs to be phosphorylated will need to be determined independently.

An issue related to determining the tyrosine phosphorylation site(s) responsible for the gating changes is to establish the mechanism by which Src associates with the NMDA receptor complex. As mentioned above, Src might bind directly to one of the receptor subunit proteins, or it might be indirectly bound via an adaptor protein. Src binding could occur through its SH3 and SH2 domains, as these are well-known to mediate protein–protein interactions. SH3 domains bind to specific proline-rich motifs [15], and Holmes *et al.* [79] have shown that Src binds to human Kv1.5 potassium channels by means of the SH3 domain. C-terminal domains of NR2 subunits contain potential consensus sequences for SH3 binding, and thus it is possible that Src may bind to NMDA receptors through

such motifs. SH2 domains bind to phosphotyrosine residues in the correct sequence context, and this is primarily determined by the amino acid residues C-terminal to tyrosine. For Src kinase, sequences containing acidic amino acids, e.g. YEEX, are preferred for SH2-mediated binding [80]. For muscle nicotinic receptors, it is known that both Src and Fyn associate with the receptor β subunit protein, and the binding of Fyn is through its SH2 domain [81]. For NMDA receptor subunits, a Src SH2 canonical consensus sequence is found in the C-terminal domain of NR-L/(X1) [34, 35]. While there are potential sequence substrates that may allow SH2 or SH3 domains of Src to bind to NMDA receptor proteins, whether they do indeed mediate the association of the kinase with the NMDA channel complex remains to be determined. Also, even if Src is indirectly associated with NMDA channel proteins, it remains possible that Src might bind by means of its SH2 and SH3 domains.

An additional region that could mediate binding of Src is the unique domain. Proteins interacting with the unique domain have been identified for Src family kinases, Hck [82] and Lyn [23]. It has also been suggested that the unique domain of Src may mediate binding of this kinase to muscle nicotinic receptors [81]. If Src binds to NMDA channels by means of the unique domain, then it is possible that this binding is occluded by the Src(40–58) peptide, which may account for the effects of this peptide. Even if amino acids 40–58 do not mediate the binding of Src to the channel complex, the effects of Src(40–58) and of the anti-src1 antibody provide strong evidence that the NMDA channel complex contains a component that interacts with this region of the unique domain of the kinase. While it is most likely that the interacting component is a protein, because the complexes are embedded in the membrane an additional possibility is that Src(40–58) might interact with a membrane lipid.

Once the target protein and site of phosphorylation are known, it is possible that the answer to the second issue raised above—how it is that phosphorylation of the target is transduced into increased NMDA channel gating—will be readily apparent. However, this is not necessarily so even if we take the simplest case, that it is one of the channel subunit proteins that is the target. It is possible that phosphorylation affects channel function primarily electrostatically, by virtue of adding negative charge at a particular spatial location, which is to say any negative charge at the particular location would have the same effect on channel function. As serine/threonine or tyrosine phosphorylation increases NMDA channel gating, the electrostatic mechanism might be a unifying explanation if the sites of phosphorylation are in close regions of the protein.

While it is possible that electrostatic considerations could be sufficient, phosphorylated tyrosine residues are known to interact with specific binding motifs, such as SH2 and PTB domains [16], in a highly-specific manner, requiring more than just electrostatic interactions. This is to say that the constraints for the functional effects on NMDA channel gating may be much greater than simply supplying negative charge to a particular amino acid or region in the protein. Tyrosine phosphorylation might recruit membrane proteins, and this recruitment may be the means for regulating channel function.

When considering how NMDA channel function may be up-regulated by phosphorylation, two alternatives present themselves: 1) that phosphorylation enhances the function of channels that are already active; and 2) that phosphorylation recruits a population of channels that are otherwise inactive in the non-phosphorylated state and that when recruited, have a higher level of activity than other channels in the patch. To discriminate between these two possibilities, we have used the non-competitive NMDA channel blocker MK-801, which is trapped by the channel and, for the duration of acute experiments, causes a permanent block of active NMDA channels. We applied MK-801 until NMDA channel activity was blocked in inside-out patches. Next we washed throughly to remove MK-801 from the solutions and then applied the Src-activating peptide or Src itself, neither of which provoked NMDA channel activity (Yu XM and Salter MW, unpublished observations). Thus, rather than recruiting dormant NMDA channels, phosphorylation by Src appears to enhance the activity of already functioning channels.

## WHAT ARE THE FUNCTIONAL CONSEQUENCES OF THE REGULATION OF NMDA RECEPTORS BY SRC?

By regulating the activity of post-synaptic NMDA receptors, tyrosine phosphorylation/dephosphorylation has the potential to modulate the efficacy of synaptic transmission. Because NMDA receptors are widely present at central synapses, there is the possibility that tyrosine phosphorylation could affect synaptic efficacy throughout the CNS. One region where Src is highly expressed is the CA1 region of the hippocampus [25]. Thus, we have examined the functional consequences of NMDA receptor regulation by Src in CA1 in an *in vitro* hippocampal slice preparation.

It is well known that tetanic stimulation of the Schaffer collateral inputs to CA1 neurons causes a persistent increase in the efficacy of synaptic transmission, known as LTP. LTP occurs at various synapses in the CNS, and it has been proposed that LTP in the hippocampus is a principal cellular substrate underlying learning and memory [83, 84]. It has been established that in the CA1 region LTP is induced by a cascade of biochemical steps occurring in the pyramidal neurons [85, 86]. Both PTK function [87] and NMDA receptor activation [88] are known to be necessary for the induction of LTP in CA1 neurons. Therefore, by using the reagents characterized previously, we explored whether Src participates in LTP [89].

We found that blocking Src with Src(40–58) or anti-src1 prevented the induction of LTP. Conversely, activating Src or administering recombinant Src induced a long-lasting potentiation of synaptic responses that occluded LTP in-

duction. Moreover, tetanic stimulation producing LTP enhanced the activity of Src, as measured *in vitro* using an immune-complex kinase assay. This enhancement of Src activity was observed at the earliest measurable timepoint, i.e. less than 1 min after the tetanic stimulation was delivered. Thus, Src is up-regulated very rapidly by the stimulation.

The principal means of expression of LTP in CA1 neurons is by enhancement of the component of synaptic responses mediated by AMPA receptors. We indeed found that the AMPA receptor-mediated synaptic responses, as well as those mediated by NMDA receptors, were potentiated by Src, an apparent contradiction to the findings described above. However, in the studies on cultured neurons intracellular $Ca^{2+}$ was highly buffered, whereas in the experiments in hippocampal slices low intracellular $Ca^{2+}$ buffering was used, as this is necessary to induce LTP. When calcium buffering in the CA1 neurons was increased, directly activating Src no longer potentiated the AMPA component of the synaptic responses, while synaptic NMDA responses were still enhanced. Thus, the enhancement of AMPA receptor-mediated synaptic responses produced by activating Src depended upon a rise in $Ca^{2+}$, but it is known that Src is not a $Ca^{2+}$-dependent enzyme [90]. This indicates that Src does not up-regulate AMPA receptors directly but rather does so indirectly through one or more steps requiring $Ca^{2+}$.

Furthermore, it was determined that Src-induced potentiation of AMPA responses is causally linked to NMDA responses because the potentiation of AMPA currents was prevented by blocking NMDA receptors. On the other hand, blocking NMDA receptors did not reverse established Src-induced potentiation of AMPA responses. Therefore, as is the case for LTP induced by tetanus, NMDA receptors are necessary to produce, but not to maintain, the potentiation of AMPA responses induced by activating Src directly.

In a recent model of LTP induction in CA1 neurons, it was postulated that during tetanus there is an influx of calcium through unblocked NMDA receptors causing a rise in intracellular [$Ca^{2+}$]. This rise in [$Ca^{2+}$] leads to activation of $Ca^{2+}$/calmodulin-dependent kinase II (CaMKII), which phosphorylates, and thereby up-regulates, the function of AMPA receptors [91, 92]. However, the new findings described above are not accounted for by this, or any other, model of LTP induction, and thus it is necessary to establish a new understanding of this process. The simplest model to account for the findings, as illustrated in Fig. 2, is that during induction of LTP Src is activated rapidly, which leads to enhanced NMDA receptor function. This enhanced NMDA receptor function boosts the entry of $Ca^{2+}$, which may thereby trigger the downstream signalling cascade. A novel aspect of this model is that the evidence suggests that LTP induction requires a previously unsuspected step upstream of NMDA receptors. It is possible that Src might also act by enhancing the biochemical steps downstream of $Ca^{2+}$ entry through NMDA receptors,

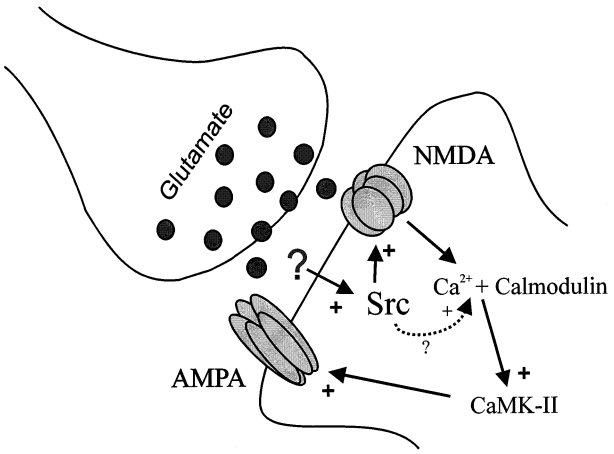


**FIG. 2. Model for the role of Src in the induction of LTP in CA1 hippocampal neurons. It is postulated that Src is activated as a consequence of tetanic stimulation and that activated Src up-regulates the function of NMDA receptors. The dashed line indicates that it is additionally possible that activated Src might act to promote the signalling cascade downstream of NMDA receptors. The transmembrane signalling mechanism leading to activation of Src remains to be determined.**

but this remains to be determined. Nonetheless, activation of Src appears to be a biochemical mechanism gating the induction of LTP in CA1 neurons.

A unique property of NMDA receptors is their voltage-dependent blockade by $Mg^{2+}$. Because depolarization removes this blockade, NMDA receptors are able to detect the coincidence of synaptic input and of post-synaptic depolarization. The studies described above indicate that NMDA receptors also function as coincidence detectors in a different way—the receptors detect the coincidence of synaptic input and of post-synaptic Src activation. As discussed next, there are numerous biochemical pathways that converge on Src. This leads to the possibility that there might be novel forms of plasticity driven by such intracellular signalling pathways rather than by neuronal depolarization *per se*.

A pressing question that follows from our recent work is—what is the mechanism causing Src activation following tetanic stimulation. There are a variety of regulatory sites on Src [14] including the C-terminal tyrosine site, which is phosphorylated specifically by the Csk family of PTKs [93, 94]. Also, a number of biochemical pathways converge to activate Src, for example through Pyk2/CAKβ and Gβγ [95, 96]. Indeed, stimulation of NMDA receptors themselves has been reported to induce tyrosine phosphorylation of neuronal proteins [97]; if this is due to enhancing Src activity, there is the potential for feed-forward up-regulation of NMDA channel function. Activation of metabotropic excitatory amino acid receptors has also been shown to cause tyrosine phosphorylation [98, 99]. Thus, there are many potential mechanisms by which Src may be activated following tetanus.

Inasmuch as the role of Src in LTP induction appears to be to enhance NMDA receptor function, then establishing

the mechanisms producing the enhancement is crucial for understanding LTP. As discussed above, one potential mechanism is via phosphorylation in one of the NMDA receptor subunit proteins. Indeed, it has been found that tyrosine phosphorylation of the NMDA receptor subunit NR2B is increased following induction of LTP in the dentate gyrus in the hippocampus [100, 101], a region where LTP induction is blocked by tyrosine kinase inhibitors [102]. The increase in phosphorylation was not detected until 5–15 min after the tetanic stimulation, which might indicate that enhanced tyrosine phosphorylation of NR2B is not involved in induction of LTP. Alternatively, it is possible that receptor phosphorylation prior to this time may have been below the detection threshold. Another CNS region where NMDA receptor-dependent synaptic plasticity has been shown to be associated with an increase in tyrosine phosphorylation of NR2B is the insular cortex. Rosenblum *et al.* [103] found that taste aversion conditioning produced a specific increase in phosphorylation of NR2B with no change in total receptor protein. While blocking NMDA receptors with 2-amino-5-phosphonovaleric acid prevented the induction of the conditioned taste aversion, it did not inhibit the increase in NR2B tyrosine phosphorylation. This contrasts with the situation in the dentate gyrus where Rostas *et al.* [101] found that the NMDA receptor antagonist MK801 prevented the tetanus-induced increase in tyrosine phosphorylation of NR2B.

While the model shown in Fig. 2 emphasizes the boost in $Ca^{2+}$ entry produced by enhancing NMDA channel gating, an additional mechanism may be that tyrosine phosphorylation of NR2B, or of the as-yet-to be-found target of Src, provides a binding site for an SH2 domain containing protein. Many proteins containing SH2 domains are signalling proteins that may come together to form signalling complexes. Thus, phosphorylation of the Src target protein could recruit a signalling complex to NMDA receptors. The recruitment may activate the complex and initiate a cascade of downstream signalling. This potential mechanism could participate, for example, in consolidation of the longest lasting phases of LTP and might contribute to the involvement of various signalling cascades such as the Ras-GAP-MAP kinase pathway [104].

A role for tyrosine kinases in induction of LTP was first suggested by O'Dell and colleagues [87] from experiments showing that LTP is blocked by bath-applied tyrosine kinase inhibitors. They subsequently reported that mutant mice in which the *src* gene had been deleted showed LTP in CA1, a genetic argument against the absolute requirement for Src in LTP induction. It was also found that LTP is blunted but not abolished in mice lacking *fyn* [105]. More recently, it has been reported that the impairment in LTP is age dependent in $fyn^-$ mice, with young $fyn^-$ animals showing LTP comparable to wild-type mice [106]. Furthermore, the impairment of LTP in the $fyn^-$ mice correlates with a large decline in the level of expression of Src. It is known that Src and Fyn may substitute for each other in various processes [107]. Therefore, while it appears that in wild-type individuals Src is a required mediator of LTP induction, in animals that develop without *src* another member of the *src* family, likely *fyn*, may substitute for *src*. This is not to say that Fyn might not also be necessary for LTP induction in the wild-type mice, but this possibility has yet to be examined using Fyn-specific reagents. Even if Fyn is involved in LTP induction, our recent results indicate that, in a linear scheme, Fyn would be upstream of Src; otherwise, the effects of the activating peptide would not have been prevented by the Src-specific blockers.

## SUMMARY

Src has been identified as an endogenous PTK that enhances the function of NMDA channels in CNS neurons. Current evidence is inconclusive as to whether the enhancement of function may be due to tyrosine phosphorylation of an NMDA receptor subunit protein or of an associated protein. A physiological consequence of the enhancement of NMDA channel function is that Src mediates induction of LTP in the CA1 region of the hippocampus. Src is expressed in various regions of the CNS [25], and in other areas, such as cerebellum [108], tyrosine phosphorylation has been implicated in synaptic plasticity. Thus, in many regions of the CNS, Src may participate in plasticity of excitatory synaptic transmission. In addition to a physiological role, the up-regulation of NMDA channel function by Src might be important in pathological conditions in which NMDA receptors have been implicated in pathogenesis, such as epilepsy, chronic pain, stroke, and neurodegeneration.

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