N-Shc and Sck, Two Neuronally Expressed Shc Adapter Homologs

THEIR DIFFERENTIAL REGIONAL EXPRESSION IN THE BRAIN AND ROLES IN NEUROTROPHIN AND Src SIGNALING*

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The Shc adapter protein is ubiquitously expressed and has been implicated in phosphotyrosine signalings following a variety of extracellular stimulation, e.g. growth factor stimulation, Ca2+ elevation, and G-protein-coupled receptor stimulation. In neuronal cells such as PC12, Shc was demonstrated to be involved in vitro in Ras-dependent mitogen-activated protein kinase activation following nerve growth factor stimulation and Ca2+ entry. However, Shc mRNA was hardly detectable in the brain, and therefore, Shc is unlikely to participate in phosphotyrosine signaling in the central nervous system. Two recently isolated Shc homologs, N-Shc and Sck, have been shown to be expressed in the brain and are expected to function as neuronal adapters instead of Shc. In this study, the neuronal distribution and function of these novel Shc members were investigated. In human and rat central nervous systems, the expression profiles of N-Shc and Sck mRNAs considerably overlapped, although some distinct localization between them was observed: in the adult rat brain, the level of N-Shc mRNA was the highest in the thalamus, whereas that of Sck mRNA was the highest in the hippocampus. In the peripheral nervous system, transcripts of Shc and Sck, but not of N-Shc, were detected. Immunoprecipitation experiments demonstrated functional differences between N-Shc and Sck: (i) N-Shc was a higher affinity adapter molecule than Sck in nerve growth factor and brain-derived neurotrophic factor signaling; and (ii) N-Shc, but not Sck, was efficiently phosphorylated by activated Src tyrosine kinase, whereas Sck, but not N-Shc, formed a complex with pp135, a protein highly phosphorylated by v-Src. These results suggest that neurally expressed N-Shc and Sck may have distinct roles in neuronal signaling in the brain.

The Shc adapter protein has been implicated in various growth factor and cytokine receptor signalings by virtue of its association with phosphotyrosine residues of the activated receptors (1, 2). Shc has two modules of phosphotyrosine recognition with different specificities, an N-terminal phosphotyrosine-binding domain (PTB domain) and a C-terminal SH2 domain (3). Shc can thereby associate with a wide variety of tyrosine kinase receptors, which phosphorylate Shc at its tyrosine residues. In addition, Shc can be phosphorylated by cytoplasmic tyrosine kinases such as Src family kinases and PYK2 kinase that mediate signaling following Ca2+ elevation and G-protein-coupled receptor stimulation (4–6). Phosphorylated Shc subsequently associates with another adapter protein, Grb2, through direct binding of the Grb2 SH2 domain to Tyr-239 on Shc (7–10). Grb2 is in turn, associated with mammalian SOS, a Ras guanine nucleotide exchange factor. Shc phosphorylation therefore induces the formation of a complex containing Shc, Grb2, and SOS, which may activate the Ras pathway (2). In neuronal cell lines such as PC12, Shc has been shown to play a pivotal role in Ras-dependent MAP kinase activation following Trk receptor stimulation with nerve growth factor (NGF) (11, 12). However, the level of Shc mRNA was found to be remarkably low in brain tissues. Recently, two Shc homologs (N-Shc/ShcC/Rai and Sck/ShcB/Sli) were isolated and shown to be expressed in the brain, and they were proposed to exert neuronal adapter functions instead of Shc (13–16).

The importance of phosphotyrosine signaling in the development and maintenance of the nervous system is widely accepted (17, 18), whereas the neuronal distribution of Shc family members has been largely unknown. Previously, we showed that N-Shc expression was brain-specific; its mRNA was detected throughout the central nervous system of the rat embryo (16). N-Shc expression in the human adult brain exhibited regional differences, and the highest levels were found in the cortex and hippocampus (16). In this study, we obtained Sck cDNA and compared its expression with that of Shc and N-Shc in neural tissues. Shc was not expressed in the brain, and some distinct localization between N-Shc and Sck was observed in the central nervous system. In contrast, only Shc and Sck mRNAs were detected in the peripheral nervous system; thus, Sck was expressed in both central and peripheral neurons, and N-Shc was confirmed to be a central nervous system-specific member of the Shc family.

In light of the distinct expression profiles of N-Shc and Sck in the brain and other neural tissues, questions arise about the possibility of their functional divergence. Shc (and other Shc members) has been implicated to provide a convergence point
for neuronal signaling pathways (19); signalings through Shc family members are not simply linear, but may branch and overlap extensively. If N-Shc and Sck have different abilities as adapter molecules, such as different levels of affinities for phosphotyrosine-containing proteins or different substrate specificities for tyrosine kinases, various neuronal signalings mediated by these Shc members might crosstalk over a wide range and in specific combinations. To assess this possibility, we compared the responses of N-Shc and Sck with epidermal growth factor (EGF), neurotrophin, and activated Src tyrosine kinase.

**Experimental Procedures**

**Cell Culture and Reagents—** COS-1, NIH3T3, and SR-3Y1 cells were maintained in Dulbecco’s modified Eagle’s /F-12 medium containing 10% fetal bovine serum. EGF and NFG were purchased from Toyobo (Osaka, Japan), and brain-derived neurotrophic factor (BDNF) came from PeproTech Inc. (London). T7-Tag monoclonal antibody was obtained from Novagen (Madison, WI); anti-phosphotyrosine monoclonal antibody 4G10 was from Upstate Biotechnology, Inc. (Lake Placid, NY); and anti-pan-Trk antibody (C-14) was from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-EGF receptor (EGFR) and anti-Grb2 antibodies were purchased from Transduction Laboratories (Lexington, KY).

**Isolation of Sck cDNA—** Several human expressed sequence tag sequences in the GenBank™ Data Bank were found to encode part of the amino acid sequence of human Sck (13). Among these expressed sequence tag sequences, 5′-CACACATGCCGCTCCTCACA-3′ in HEEB09 and 5′-TGGTGGACTGCTTCGACCAAGGA-3′ in T06885 were selected as primers to produce an 810-bp Sck probe by reverse transcription-polymerase chain reaction with poly(A)-containing RNA isolated from human brain. A human frontal cortex-derived cDNA library (Stratagene, La Jolla, CA) was screened with this Sck probe. Eight overlapping cDNAs were obtained, and their sequences were determined. Thereafter, a 470-bp HindIII-HindIII fragment of the clone located closest to the 5′-end of Sck mRNA was isolated and used as a probe for further screening. Six additional clones were obtained, and their composite nucleotide sequence was determined. The rat homolog of human Sck was isolated by screening a rat brain cDNA library (Stratagene) with the human 810-bp Sck cDNA fragment as a probe. Four overlapping clones were obtained, and their composite nucleotide sequence was determined.

**Northern Blot Analysis—** Human multiple tissue blots I and II and brain tissue blot II and III (CLONTECH, Palo Alto, CA) were hybridized with the nucleotide sequence of Sck cDNA, which had a complete encoding capacity for PTB, CH, and SH2 domains. To elucidate the regulatory distribution of Shc family members in the nervous system, we sought to obtain Sck cDNA in addition to the previously isolated Shc and N-Shc cDNAs (16). Since the nucleotide sequence of Sck was not reported, we amplified an 810-bp Sck fragment by reverse transcription-polymerase chain reaction based on expressed sequence tag sequences encoding partial amino acid sequences of human Sck protein (13). By screening a human frontal cortex-derived cDNA library with this probe, we obtained 14 overlapping clones. The determined nucleotide sequence of human Sck cDNA is 2390 bp in length and has an open reading frame of at least 1470 nucleotides and a poly(A) tail at its 3′-end. The deduced Sck amino acid sequence includes complete PTB, CH, and SH2 domains, although the N terminus of the encoded protein could have been slightly deleted (Fig. 1). By Northern blot analysis, the major transcript of human Sck was estimated to be ∼2.7 kilobases (Fig. 2), suggesting that ∼300 bp or so of the 5′-end were missing from these clones. Fig. 2 also shows the existence of minor isoforms of Sck transcripts. Some differences at the N terminus were found between this human Sck sequence and that previously reported by Pellici et al. (15). The human Sck sequence in Fig. 1 and that reported by Kavanaugh and Williams (13) were the same in the range that we can compare, whereas the sequence reported by Pellici et al. was different from that reported by Kavanaugh and Williams as well as from our sequence at the N terminus. Thus, we believe that our sequence is correct. The discrepancy of the reported N-terminal sequences of Sck protein may come from the alternatively spliced transcripts described above. We also isolated rat Sck cDNA, which had a complete encoding capacity for PTB, CH, and SH2 domains.

These nearly full-length Sck clones revealed that the N-terminal PTB and the C-terminally located SH2 domains are highly homologous to those of Shc and N-Shc (Fig. 1). In contrast, the internal CH domain of Sck diverges from the others, although there are several conserved residues that are functionally important (20), e.g., (i) YVN(T/V) (residues 321–324 in human Sck and residues 299–302 in rat Sck), the Grb2-binding site; (ii) YXXY (residues 243–247 in human Sck and residues 218–222 in rat Sck), which was shown to act as another Grb2-binding site (9, 10); and (iii) GR(l)DFDM(K/R)FPE (residues 321–324 in human Sck and residues 321–324 in rat Sck), which was required in Shc for adaptin binding (26).
there were several proline-rich motifs that were unique to each Shc member (Fig. 1). These proline-rich motifs are considered to be the recognition sites of SH3-containing proteins (27). A comparison of human and rodent Sck, N-Shc, and Shc revealed that each Shc member has unique potential SH3-binding site(s), thus leading to differential downstream signalings.

Tissue Distribution of Shc, N-Shc, and Sck Transcripts—We compared Sck expression with that of Shc and N-Shc using the same blot of human adult tissues (Fig. 2A). As previously reported, Shc was broadly expressed in most human tissues except brain. In marked contrast, N-Shc mRNA was specifically expressed in brain tissue and was hardly detectable in other tissues except pancreas. Similar to Shc mRNA, the Sck message was distributed rather broadly. Notably, the brain showed a moderate level of Sck mRNA expression. Thus, Sck is the second major member of the Shc family that is expressed in the human brain.

Regional Expression of N-Shc and Sck mRNAs in the Brain—We next compared the expression of the Shc, N-Shc, and Sck mRNAs in various brain regions and spinal cord in human by Northern blot analysis (Fig. 2B). The distribution of N-Shc and Sck mRNAs in the human brain was rather complex. N-Shc mRNA was detected in abundance in the cerebral cortex, frontal and temporal lobes, occipital pole, hippocampus, caudate nucleus, and amygdala, but its level was very low or undetectable in the cerebellum, medulla, and spinal cord. Expression of Sck mRNA overlapped partially with that of N-Shc mRNA, with notable differences: N-Shc mRNA was highly expressed in the caudate nucleus and was very low in the hypothalamus, whereas Sck mRNA was abundantly expressed in the hypothalamus and was nearly undetectable in the caudate nucleus (Fig. 2B).

To further examine the regional expression of N-Shc and Sck transcripts in the brain, we compared the mRNA expression of the Shc members in rat brain tissues by in situ hybridization. In our previous experiments, N-Shc mRNA was found to be rather broadly expressed in the embryonic nervous system and the brain (16). Surprisingly, however, in the mature brain, it became evident that the N-Shc message was particularly abundant in the thalamus (Fig. 3B). In contrast, Sck mRNA was widely distributed throughout the whole brain, with the highest levels found in the hippocampus (Fig. 3C). In a high-power view of the hippocampal area, both N-Shc and Sck mRNAs were detected in both pyramidal neurons and...
granule cells in the CA1 through CA3 regions and dentate gyrus, respectively; cells in the hilus also showed high expression of N-Shc and Sck messages (data not shown).

Although only N-Shc and Sck were detected in the brain, this was not true for the peripheral nervous system, where a considerable level of Shc mRNA expression was observed (Fig. 4). Shc transcripts were predominantly observed in the superior cervical ganglion (SCG) areas (Fig. 4A). Shc-positive cells were only sparsely distributed in the dorsal root ganglion (DRG). Shc mRNA expression in the spinal cord was restricted to part of the motor neurons (Fig. 4A, small arrow). Sck mRNA expression in the peripheral nervous system (DRG and SCG) was much higher than in the brain (Fig. 4C). Although the Sck message was broadly detected in the spinal cord, high-level expression was restricted to the motor neurons. In contrast, N-Shc expression in DRG and SCG was very low or undetectable (Fig. 4B). In the spinal cord, an overall expression of N-Shc mRNA was observed.

The regional expression profile of N-Shc and Sck mRNAs in the adult rat brain (Fig. 3) was considerably different from that observed when RNA samples extracted from post-mortem human brain were analyzed by Northern blotting (Fig. 2B). Consistent with in situ data, semiquantitative reverse transcription-polymerase chain reaction experiments using RNA samples from various rat brain regions demonstrated that the thalamus was the highest source of N-Shc mRNA. The striatum and cerebral cortex showed moderate levels of N-Shc mRNA expression, and the cerebellum showed a low level of N-Shc mRNA expression (data not shown). Thus, the apparent difference in the expression profiles of N-Shc and Sck mRNAs may reflect a species variation.

EGF Signaling Is Equally Mediated by Both N-Shc and Sck—Given the structural similarity and differential expression profile, questions arise as to how N-Shc and Sck share or differentiate neuronal signaling pathways. Also, do their adapter functions overlap with each other, or do they have certain distinct roles? We sought to answer these questions by examining the binding affinities of N-Shc and Sck for EGF and neurotrophin receptors as well as by investigating the response of N-Shc and Sck to the action of Src, a non-receptor tyrosine kinase.

We previously demonstrated that activated EGFR was able to bind N-Shc and to induce N-Shc tyrosine phosphorylation and subsequent association with Grb2 (16). Since we obtained a nearly full-length Sck cDNA that encoded complete PTB, CH, and SH2 domains, we asked the question of whether Sck is similarly responsive to EGF signaling compared with Shc and N-Shc. We performed a co-immunoprecipitation experiment using T7 peptide-tagged N-Shc or Sck and compared their responses to EGF stimulation. Even though the T7 peptide-tagged Sck cDNA used here lacked the most 5'-end of the open reading frame, the deduced Sck amino acid sequences contained complete PTB, CH, and SH2 domains. Therefore, we believe that the expressed Sck retains full activity concerning tyrosine phosphorylation pathways, as has been demonstrated for p46Shc, the smallest Shc isoform (13, 28, 29). COS-1 cells were transfected with either T7 peptide-tagged N-Shc or Sck cDNA and were then stimulated with EGF for 5 min, lysed, and immunoprecipitated with anti-T7 peptide antibody. The immunoprecipitates were then immunoblotted with anti-EGFR antibody. EGF stimulated equally well the association between EGFR and N-Shc and between EGFR and Sck (Fig. 5A). In addition, a similar increase was observed in the tyrosine phosphorylation of N-Shc and Sck (Fig. 5B). Furthermore, the subsequent binding of either N-Shc or Sck to Grb2 was nearly identical (Fig. 5C). These results indicate that EGF signaling is mediated efficiently by both N-Shc and Sck in a quantitatively similar manner.

N-Shc Has Higher Affinity for TrkA and TrkB Receptors than Sck—We next compared the responses of N-Shc and Sck fol-

![Fig. 2. Tissue and neuronal distribution of human She family members. A, Northern blot of Shc (upper), N-Shc (middle), and Sck (lower) mRNAs from various human adult tissues. Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas; lane 9, spleen; lane 10, thymus; lane 11, prostate; lane 12, testis; lane 13, ovary; lane 14, small intestine; lane 15, colon; lane 16, peripheral blood leukocyte. B, Northern blot of Shc (upper), N-Shc (middle), and Sck (lower) mRNAs from various areas of adult human brain. Lane 1, cerebellum; lane 2, cerebral cortex; lane 3, medulla; lane 4, spinal cord; lane 5, occipital pole; lane 6, frontal lobe; lane 7, temporal lobe; lane 8, putamen; lane 9, amygdala; lane 10, caudate nucleus; lane 11, corpus callosum; lane 12, hippocampus; lane 13, hypothalamus; lane 14, substantia nigra; lane 15, subthalamic nucleus; lane 16, thalamus.](http://www.jbc.org/)

![Fig. 3. In situ hybridization showing the distribution of Shc, N-Shc, and Sck mRNAs in the adult rat brain. Representative autoradiograms of in situ hybridization experiments using nearly adjacent horizontal sections and 35S-labeled antisense RNA as a probe for each member of the Shc family are shown. A, Shc probe; B, N-Shc probe; C, Sck probe. Experiments using the sense control showed only background levels of signals (not shown), as shown in A for Shc. The arrowhead in B points to the thalamus, and the small arrow points to the hippocampus. Th, thalamus; Hp, hippocampus; Ob, olfactory bulb. Scale bar = 5 mm.](http://www.jbc.org/)
following NGF or BDNF stimulation by using transfected fibroblasts (NIH3T3) as a model (Fig. 6, A–J). NIH3T3 cells were transfected with either T7 peptide-tagged N-Shc or Sck cDNA together with a Trk-expressing plasmid. The transfected cells were incubated for 5 min with or without neurotrophins (NGF or BDNF), lysed, and immunoprecipitated with anti-T7 peptide antibody. The immunoprecipitates were then immunoblotted with anti-pan-Trk antibody. In these experiments, N-Shc was efficiently associated with TrkA and TrkB receptors in the presence or absence of ligands. Surprisingly, however, Sck was unable to form such a complex with Trk receptors under these conditions (Fig. 6, A and F). The T7 peptide-tagged N-Shc and Sck proteins recovered from the transfected cells were equal in amount (Fig. 6, D and I). More important, even in the transfection with Sck cDNA, the levels of Trk expression were nearly equal as evidenced by the immunoblotting of whole lysates with anti-Trk antibody (Fig. 6, E and J). The different affinities of N-Shc and Sck for Trk receptors were confirmed by another immunoprecipitation experiment with anti-Trk antibody using the similarly transfected COS-1 cells (Fig. 6, K–M). Anti-Trk immunoprecipitates contained a large amount of N-Shc, although the Sck protein in these immunoprecipitates was nearly undetectable (Fig. 6L). Furthermore, Fig. 6 (B and G) shows that the tyrosine phosphorylation of N-Shc by activated Trk receptors was more efficient than that of Sck. In addition, the subsequent binding of N-Shc to Grb2 was higher than that of Sck (Fig. 6, C and H). However, the tyrosine phosphorylation of Sck and the association between Sck and Grb2 were somewhat detectable, and the degrees of Sck phosphorylation and Sck-Grb2 binding were significantly increased in the presence of ligand (Fig. 6, B, C, G, and H). There may be a functional difference between the phosphorylated N-Shc associated with Trk receptors and the phosphorylated Sck separate from the Trk receptors (see “Discussion”). In these experiments, the induction by neurotrophins of the association between Trk receptors and N-Shc/Sck as well as the subsequent phosphorylation and Grb2 binding of N-Shc/Sck were moderate; this could be mainly due to the overexpression of Trk receptors (see “Discussion”). The co-immunoprecipitation experiments using T7 peptide-tagged N-Shc and Sck proteins thus revealed that N-Shc is a higher affinity adapter protein than Sck for TrkA and TrkB receptors, although a different role of phosphorylated Sck in neurotrophin signaling can be considered.

A Divergent Response between N-Shc and Sck to Src Tyrosine Kinase—A more marked functional difference between N-Shc and Sck was found in the response to Src tyrosine kinase. We transfected v-src-transformed rat 3Y1 fibroblasts (SR-3Y1) with T7 peptide-tagged N-Shc or Sck cDNA. Transfected cells were serum-starved, lysed, and immunoprecipitated with anti-T7 peptide antibody. The immunoprecipitates were then immunoblotted with antibodies as indicated: anti-EGFR antibody (A), anti-phosphotyrosine (anti-PTyr) antibody (B), and anti-Grb2 antibody (C). (D), to confirm that equal amounts of N-Shc and Sck proteins were immunoprecipitated with anti-T7 peptide antibody, the same immunoprecipitates were immunoblotted with anti-T7 peptide antibody.
tion of Sck was hardly detectable (Fig. 7A). The T7 peptide-tagged N-Shc and Sck proteins recovered from the transfected SR-3Y1 cells were equal in amount (Fig. 7B). Another important finding is that Sck specifically associated with a particular tyrosine-phosphorylated protein of ~135 kDa (designated pp135) in SR-3Y1 cells (Fig. 7A). N-Shc-pp135 binding was less tight by at least 20-fold. In the course of analyzing the neurotrophin signaling, we also observed the binding of Sck to pp135, although it was very weak; a prolonged exposure of the blots presented in Fig. 6 (B and G) revealed the association between Sck and pp135 (Fig. 7, C and D). Sck-pp135 binding was independent of neurotrophin/Trk (Fig. 7, C and D) as well as EGF (data not shown) stimulation; thus, the tyrosine phosphorylation of pp135 in Fig. 7 (C and D) can be regarded as basal. These data indicate that pp135, a possible partner of Sck, is highly and specifically phosphorylated by Src tyrosine kinase.

DISCUSSION

The Shc adapter protein functions in mediating a variety of phosphotyrosine signalings (2). In the past few years, we and others have isolated two additional Shc-related sequences, i.e. N-Shc/ShcC/Rai (14–16) and Sck/ShcB/Sli (13–15), both of which are expressed in the brain. Thus, shc, N-shc, and sck form a small gene family, the shc family; however, N-Shc and Sck are the dominant adapters in the brain. In this study, we demonstrated distinct expression profiles of the three Shc family members in the central and peripheral nervous systems as well as differential responses of N-Shc and Sck to neurotrophin signaling and to Src activation.

If differential expression in the Shc family means distinct roles of the respective members, their localization should give us good clues for elucidating their functions. For example, high-level expression of N-Shc transcripts in the adult rat brain was restricted to the thalamus. It is of interest to note that another molecule in cytoplasmic signaling, protein kinase Cδ, was recently shown to be highly and specifically expressed in the thalamus (30). Protein kinase Cδ can be tyrosine-phosphorylated by Src tyrosine kinase. If differential expression in the Shc family means distinct roles of the respective members, their localization should give us good clues for elucidating their functions. For example, high-level expression of N-Shc transcripts in the adult rat brain was restricted to the thalamus. It is of interest to note that another molecule in cytoplasmic signaling, protein kinase Cδ, was recently shown to be highly and specifically expressed in the thalamus (30). Protein kinase Cδ can be tyrosine-phosphorylated by Src tyrosine kinase. If differential expression in the Shc family means distinct roles of the respective members, their localization should give us good clues for elucidating their functions. For example, high-level expression of N-Shc transcripts in the adult rat brain was restricted to the thalamus. It is of interest to note that another molecule in cytoplasmic signaling, protein kinase Cδ, was recently shown to be highly and specifically expressed in the thalamus (30). Protein kinase Cδ can be tyrosine-phosphorylated by Src tyrosine kinase. If differential expression in the Shc family means distinct roles of the respective members, their localization should give us good clues for elucidating their functions. For example, high-level expression of N-Shc transcripts in the adult rat brain was restricted to the thalamus. It is of interest to note that another molecule in cytoplasmic signaling, protein kinase Cδ, was recently shown to be highly and specifically expressed in the thalamus (30). Protein kinase Cδ can be tyrosine-phosphorylated by Src tyrosine kinase. If differential expression in the Shc family means distinct roles of the respective members, their localization should give us good clues for elucidating their functions. For example, high-level expression of N-Shc transcripts in the adult rat brain was restricted to the thalamus. It is of interest to note that another molecule in cytoplasmic signaling, protein kinase Cδ, was recently shown to be highly and specifically expressed in the thalamus (30). Protein kinase Cδ can be tyrosine-phosphorylated by Src tyrosine kinase. If differential expression in the Shc family means distinct roles of the respective members, their localization should give us good clues for elucidating their functions. For example, high-level expression of N-Shc transcripts in the adult rat brain was restricted to the thalamus. It is of interest to note that another molecule in cytoplasmic signaling, protein kinase Cδ, was recently shown to be highly and specifically expressed in the thalamus (30). Protein kinase Cδ can be tyrosine-phosphorylated by Src tyrosine kinase.
of NGF (33, 41). These results may explain why the marginal induction by neurotrophins was observed in Fig. 6. In fact, a marked increase in the tyrosine phosphorylation of N-Shc following BDNF stimulation was observed in primary cultures of rat cerebral cortical neurons.²

The neuronal function of Shc has recently been extended to be involved in the signalings activated by calcium elevation and G-protein-coupled receptor stimulation (6, 42). Elevation of the Ca²⁺ level and G-protein-coupled receptor stimulation were shown to induce Ras-dependent MAP kinase activation and were suggested to be mediated by Src family kinases, a brain-enriched PYK2 tyrosine kinase, and EGFR (5, 43–45). These tyrosine kinases were shown to phosphorylate Shc and were suggested to activate the Ras/MAP kinase pathway by virtue of Shc and the Grb2/SOS complex (6, 19). In the central nervous system, however, we now have two Shc homologs, N-Shc and Sck. N-Shc and Sck were shown to mediate EGFR signaling equally (Fig. 5). Therefore, both adapter proteins could be utilized in the neuronal signals by virtue of the tyrosine kinase activity of EGFR following calcium entry and neurotransmitter stimulation.

We found that N-Shc, but not Sck, was efficiently phosphorylated by activated Src tyrosine kinase, whereas Sck, but not N-Shc, associated with pp135, which appeared to be highly phosphorylated by v-Src. The pp135 binding may be Sck-specific because no similar tyrosine-phosphorylated protein was coprecipitated with anti-Shc antibody in v-src-transformed rat fibroblasts (4). Src was found to be involved in Ras-dependent MAP kinase activation following Ca²⁺ entry in PC12 cells (43). Src and other Src family members, e.g. Fyn and Lyn, were recently shown to mediate the signals from G-protein-coupled receptors to Ras/MAP kinase by way of the Shc-Grb2/SOS complex (44, 46, 47). The notable difference between N-Shc and Sck in the response to activated Src kinase indicates distinct functions of these two adapter molecules in signaling cascades mediated by Src (or Src family kinases) in central neurons.

Recently, Shc (and the Shc family) has been discussed to provide a convergence point for multiple signaling pathways: one path is composed of receptor tyrosine kinases, adapter proteins, and Ras, and the other pathway uses calcium and trimeric G-proteins (6, 19). The presumed role of Shc family members as go-betweens in the signaling crosstalk seems more plausible in the nervous system in light of the observations that Ca²⁺-dependent survival of cortical neurons can be blocked by anti-BDNF antibody (48), and cultured retinal ganglion cells respond to growth factor stimulation more efficiently when depolarized (49). Based upon our new findings of distinct expression profiles and functional specificities of N-Shc and Sck, we assume that multiple signalings in the central nervous system mediated by N-Shc and Sck can crosstalk in a specific combination.

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