Agonist-dependent Interaction of the Rat Somatostatin Receptor Subtype 2 with Cortactin-binding Protein 1*

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We report here an interaction between the C terminus of the rat somatostatin receptor subtype 2 (SSTR2) and a protein that has recently been identified as cortactin-binding protein 1 (CortBP1). Interaction is mediated by the PDZ (PSD-95/discs large/ZO-1) domain of CortBP1. As shown by in situ hybridization, SSTR2 and cortactin-binding protein are coexpressed in the rat brain. The association between SSTR2 and the PDZ-domain of CortBP1 was verified by overlay assays and by coprecipitation after transfection in human embryonic kidney (HEK) cells. Analysis by confocal microscopy indicates that CortBP1 is distributed diffusely throughout the cytosol in transfected cells and that it becomes concentrated at the plasma membrane when SSTR2 is present. This process is largely increased when the receptor is stimulated by somatostatin; as CortBP1 interacts with the C terminus of SSTR2, our data suggest that the binding of agonist to the receptor increases the accessibility of the receptor C terminus to the PDZ domain of CortBP1. Our data for the first time establish a link between a G-protein coupled receptor and constituents of the cytoskeleton.

Neuronal receptors for chemical transmitters are not uniformly distributed throughout the cell but are concentrated at specific cellular sites; this is particularly true for receptors belonging to the group of ligand-gated ion channels, which are clustered at postsynaptic sites through interaction with specific proteins such as rapsyn (1), gephyrin (2), or the PSD/SAP proteins (3). In contrast, very little is known about proteins specifically distributed throughout the cell but are concentrated at the plasma membrane when SSTR2 is present. This process is largely increased when the receptor is stimulated by somatostatin; as CortBP1 interacts with the C terminus of SSTR2, our data suggest that the binding of agonist to the receptor increases the accessibility of the receptor C terminus to the PDZ domain of CortBP1. Our data for the first time establish a link between a G-protein coupled receptor and constituents of the cytoskeleton.

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The abbreviations used are: GPCR, G-protein-coupled receptor; CortBP1, cortactin-binding protein 1; GST, glutathione S-transferase; HEK, human embryonic kidney; PBS, phosphate-buffered saline; PDZ, PSD-95/discs large/ZO-1; PSD, postsynaptic density; SAP102, synapse-associated protein 102; SST, somatostatin; SSTR, somatostatin receptor.
ice, and 20-μm sections were prepared on a cryostat (Leitz, Wetzlar, Germany). Anti-sense RNA probes labeled with α-35S-UTP (NEN Life Science Products) were generated using the rat SSTR2 CDNA (14) and a rat cortactin-binding protein clone obtained from Dr. J. T. Parsons (University of Virginia, Charlottesville, VA). In situ hybridization experiments were performed as described (16). Sections were exposed to BioMax MR film for 3 days.

Expression in HEK Cells and Coimmunoprecipitation—The cDNAs for SSTR2 carrying an N-terminal TT7-tag (17, 18) and CortBP1 (donated by Dr. J. Thomas Parsons) were coexpressed in HEK cells by transient transfection using the calcium phosphate method (19). Cells were lysed in radioimmune precipitation buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM Tris-HCl, pH 7.4), and the epitope-tagged receptor was precipitated from the supernatant fraction using the monoclonal TT7-antibody as described (18). Precipitates were analyzed by Western blotting; CortBP1 was detected using the anti-PDZ domain antibody as a primary antibody and goat-anti-rabbit IgG coupled to alkaline phosphatase (Santa Cruz Biotechnology Inc., Santa Cruz, CA) as secondary antibody. The efficiency of the precipitation was checked by immunoblotting using an anti-SSTR2 polyclonal antiserum (antiserum 6291, donated by Dr. Stefan Schulz, Magdeburg, Germany) as primary antibody.

Confocal Microscopy—For the colocalization of SSTR2 and CortBP1, transfected HEK cells were plated on poly-d-lysine-coated glass coverslips. After incubation in normal growth medium for 3 days, cells were fixed using 4% paraformaldehyde in phosphate-buffered saline (PBS) as described (17) and permeabilized in 0.1% Triton X-100 in PBS for 90 s. After incubation with a mixture of T7 monoclonal antibody and the 189.3 rabbit polyclonal antiserum (1:5,000 in PBS containing 2% normal goat serum) overnight at 4°C, cy2-conjugated goat-anti-mouse and cy3-conjugated goat-anti-rabbit were used as secondary antibodies (18). Cells were viewed with a confocal microscope (Leica laser scanning microscope) using specific settings for cy2 (argon laser/excitation at 488 nm/emission band pass 510–525 nm) and cy3 (helium-neon laser/excitation at 543 nm/emission low pass 570 nm). The absence of bleed-through between the two fluorophores was verified by performing parallel staining experiments on cells expressing either one or the other antigen only.

RESULTS

Identification of SSTR2-interacting Proteins—The intracellular C-terminal tail of the rat SSTR2 was used as bait in a yeast two-hybrid screen of a human brain cDNA library. In this screen five positive clones were obtained, which were identified as the human isoform of synapse-associated protein 102 (SAP102, three independent clones; Ref. 20), syntrophin a1 (21) and a novel protein fragment (clone 16), which contains a PDZ domain that is highly homologous (89% identity) to the PDZ domain of the rat cortactin-binding protein 1 (CortBP1; Ref. 22; see Fig. 1A). As SAP102 and syntrophin a1 also contain PDZ domains, these results indicated a preference of the SSTR2 C terminus for interactions with PDZ domains. To identify which of these proteins might indeed interact with the somatostatin receptor subtype 2, GST fusion proteins of the PDZ domains of SAP102, syntrophin and CortBP1, were expressed and purified in E. coli. Binding to the SSTR2 C terminus was analyzed in an overlay assay using a biotinylated SSTR2 fusion protein as a probe (20). In this assay, the full-length PDZ domain of CortBP1 and some smaller proteolytic fragments thereof elicited a strong response (Fig. 1B). The strong reaction by the smaller fragments (which appear to be only minor contaminants when judged from the Coomassie stained gel) may reflect a better ability of these fragments to refold after SDS-gel electrophoresis. In contrast to CortBP1, the PDZ domains of SAP102 and syntrophin showed no reaction (Fig. 1B). No response was also observed when the overlay reaction was performed with a biotinylated control protein derived from the empty pinpoint Xa3 vector (data not shown). These results suggest that the interaction between the C terminus of SSTR2 and the PDZ domain of the CortBP1 is of high affinity, whereas the interaction with the other proteins containing PDZ domains is of low affinity, the latter of which were obviously insufficient to obtain a positive signal in the yeast two-hybrid screen but are not likely to be of physiological significance. To analyze whether SSTR2 and CortBP1 are coexpressed in the same regions of the rat brain, in situ hybridization experiments were carried out. A striking overlap between the expression pattern of the mRNAs coding for SSTR2 and CortBP1 was observed. Prominent expression of both mRNAs was detected in the cortex, the CA1, CA2, and CA3 regions of the hippocampus and in the medial habenula (Fig. 2). No labeling was observed in control sections that had been hybridized with sense RNA probes (data not shown).

CortBP1 Interacts with the SSTR2 when Expressed in HEK Cells—CortBP1 was coexpressed with an epitope-tagged version of the rat SSTR2 in HEK cells (Fig. 3). The T7-tag was introduced at the N terminus of the receptor in order to avoid interference with the PDZ-binding motif at the receptor C terminus. When the receptor was precipitated from cellular extracts using an anti-T7 monoclonal antibody, Western blotting analysis could readily detect CortBP1 in the precipitate. In a control experiment, the 180-kDa band specific for CortBP1 was not observed when cells were transfected with CortBP1 only (Fig. 3).

The cellular location of CortBP1 was examined in HEK cells transiently transfected with either CortBP1 alone or with SSTR2 and CortBP1 (Fig. 4). In the absence of SSTR2 CortBP1 exhibits an exclusively cytoplasmic staining pattern. The nucleus is spared, and there is no colocalization of CortBP1 at the plasma membrane. When cDNA coding for SSTR2 is added to the transfection mixture, fluorescence label specific for the receptor is detectable at the cell surface, and only limited intracellular staining can be observed. In these cells, the subcellular localization of CortBP1 undergoes a significant change, as it becomes concentrated at the cell surface. This effect is somewhat variable, as illustrated by the two examples in Fig.

FIG. 1. Interaction of CortBP1 with the SSTR2 C terminus. A, sequence alignment of the human PDZ-domain protein fragment and CortBP1. Shown is a sequence alignment between the PDZ domains of the human fragment (clone 16) obtained by the yeast two-hybrid screen and CortBP1. Identical amino acids are denoted by vertical bars and similar amino acids by dots. B, overlay assay. GST fusion proteins of the PDZ domains of proteins detected by the yeast two-hybrid screen were run on SDS-polyacrylamide gels and either stained with Coomassie Brilliant Blue (left panel) or blotted onto nitrocellulose membranes and probed with the biotinylated fusion protein containing the SSTR2 C terminus (right panel). Specifically bound fusion protein was detected using alkaline phosphatase coupled to avidin. SAP102 R123 contains all three domains, but SAP102 R1 only the first PDZ domain.
obtained from the center of the cells. In Cells were examined by confocal microscopy, and optical sections were directed against CortBP1 and the T7-tag at the N terminus of SSTR2.

from the cy2/SSTR2-specific channel (upper panels) fluorescence is shown, whereas in

4; in some cells there is still substantial intracellular staining for CortBP1 and only a moderate increase of surface staining. In others, almost exclusive staining at the surface can be observed. This may depend on the relative expression levels of both proteins in individual cells. However, surface staining for CortBP1 becomes much more prominent in cells that were treated with somatostatin for 5 min. Under these conditions some receptors already start to migrate into intracellular vesicles due to agonist-dependent endocytosis (18), whereas the majority of the receptor-specific fluorescence is still located on the cell surface. In all cells examined that did coexpress SSTR2 and CortBP1, CortBP1 exhibited strong plasma membrane staining when the receptor had been stimulated with agonist. These data suggest that the C terminus of the rat SSTR2 is involved in the structural rearrangements induced by the agonist. 

**DISCUSSION**

In this report we have identified a protein that interacts specifically with the C terminus of one of the five somatostatin receptor subtypes, SSTR2. CortBP1 is abundantly expressed in the mammalian central nervous system in a pattern showing strong overlap with the expression pattern of SSTR2, suggesting that the interaction observed is likely to occur in a wide variety of neuronal cell types.

Interaction between SSTR2 and its binding protein is likely to be directed by the receptor C terminus, as the C-terminal sequence of SSTR2 (Q-T-S-I-Stop) contains the consensus sequence for recognition of PDZ domains, i.e., (S/T/X)-(V/I)-Stop, where X can be any amino acid residue (3, 23). Indeed the interaction between the SSTR2 bait and its target obtained in the yeast screen is abolished when the C terminus is mutated (data not shown).

CortBP1 is a multidomain protein that contains multiple SH3-binding sites and a SAM domain; CortBP1 interacts with the cytoskeletal protein cortactin via the SH3-domain of cortactin and is thus linked to cortical actin filaments (22). Therefore we propose that the interaction of SSTR2 with CortBP1 provides a physical link between a G-protein-coupled receptor and the cytoskeleton, which could be important for immobilizing the SSTR2 at specific sites in the membrane. It is noteworthy that CortBP1 (and cortactin) was prominently detected in the axonal growth cones of hippocampal neurons in culture (22). Thus CortBP1 could be responsible for anchoring SSTR2 in nerve terminals, where it has been localized by immunocytochemistry in rat brain and where it functions as a presynaptic receptor in the regulation of transmitter release via the inhibition of N-type calcium channels (10, 24).

In transfected cells, CortBP1 undergoes a dramatic relocalization to the plasma membrane if SSTR2 is cotransfected. This effect is enhanced when the receptor is stimulated with agonists, suggesting that an increased proportion of the receptor becomes accessible at its C terminus. A similar effect has been observed before (6) demonstrating that the C terminus of the β-adrenergic receptor interacts with a PDZ domain of the regulatory factor of the Na+/H+ exchanger only if the receptor is stimulated by agonists. This interaction leads to transduction of a signal from the receptor to the exchanger protein (6). On a structural level, these and our data imply that the C-terminal intracellular tail of G-protein-coupled receptors is somehow involved in the structural rearrangements that take place after activation.

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**Somatostatin Receptor/PDZ Domain Interaction**

**FIG. 1.** Association of SSTR2 with CortBP1 in transfected HEK cells. Cells expressing either CortBP1 (A) or CortBP1 and SSTR2 (B) were stained with antibodies directed against CortBP1 and the T7 tag at the N terminus of SSTR2. The first two lanes show Western blots from cellular lysates using the 189.3 antiseraum which recognizes CortBP1. The two right lanes show Western blots of immunoprecipitates (IP) where the SSTR2 was precipitated from cellular lysates using the T7 antibody directed against the epitope tag at the N terminus of the receptor. Note the absence of staining in lanes where SSTR2 cDNA was omitted from the transfection mixture.

**FIG. 2.** Comparison of the expression patterns of SSTR2 and CortBP1 in the rat brain. Adjacent coronal sections from frozen adult rat brains were probed with 35S-labeled antisense cRNA of rat SSTR2 (A) or rat CortBP1 (B). Cx, cortex; Hi, hippocampus; Mhb, medial habenula.

**FIG. 3.** Association of SSTR2 with CortBP1 in transfected HEK cells. HEK cells were transiently transfected with expression vectors for rat CortBP1 either alone or in combination with Ntag-SSTR2. The first two lanes show Western blots from cellular lysates using the 189.3 antiseraum which recognizes CortBP1. The two right lanes show Western blots of immunoprecipitates (IP) where the SSTR2 was precipitated from cellular lysates using the T7 antibody directed against the epitope tag at the N terminus of the receptor. Note the absence of staining in lanes where SSTR2 cDNA was omitted from the transfection mixture.

**FIG. 4.** Colocalization in HEK cells. Cells expressing either CortBP1 (A) or CortBP1 and SSTR2 (B) were stained with antibodies directed against CortBP1 and the T7-tag at the N terminus of SSTR2. Cells were examined by confocal microscopy, and optical sections were obtained from the center of the cells. In A only the CortBP1-specific fluorescence is shown, whereas in B fluorescence signals were recorded from the cy2/SSTR2-specific channel (upper panels) and the cy3/ CortBP1 specific channel (lower panels). Arrows indicate the positions where fluorescence specific for CortBP1 is enriched at the plasma membrane; arrowheads point at the position of endocytotic vesicles containing SSTR2.
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