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β-Arrestin is involved in the desensitization but not in the internalization of the somatostatin receptor 2A expressed in CHO cells

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Abstract The interaction of β-arrestin-1 with the somatostatin receptor type 2A (sst2A) was monitored using both biochemical and confocal imaging approaches. We show that, using transient transfection of either β-arrestin-1 or its dominant negative Δ-arrestin-1 in CHO cells stably transfected with the sst2A, β-arrestin-1 is colocalized with the receptor in endosomal vesicles after somatostatin-induced sequestration. However, this interaction leads to a role of β-arrestin-1 in the desensitization of the sst2A rather than in the internalization process of the receptor–ligand complex.

Key words: Somatostatin; Receptor; β-Arrestin; Internalization; Desensitization

1. Introduction

Somatostatin (somatotropin release inhibitory factor, SRIF) exerts endocrine, exocrine and neuronal functions through activation of six known G protein-coupled receptors (GPCR) (sst1, sst2A, sst2B, sst3, sst4 and sst5) [1,2]. All these receptors couple to Gi/Go proteins and are able to internalize with various efficiencies after binding of the peptide [3,4]. Signal transduction mechanisms activated by SRIF–receptor interaction involve both pertussis toxin-sensitive and -insensitive G proteins. Thus, the inhibition of adenylate cyclase and Ca^{2+} channels as well as the stimulation of K^+ channels, phospholipase C, phosphatases, arachidonic acid release, and mitogen-activated protein kinases observed after activation of sst receptors are inhibited by pertussis toxin [5–12], whereas inhibition of the SRIF-induced Na/H exchange is insensitive to the toxin [13].

A common property of most GPCRs is that ligand treatment decreases receptor responsiveness through a mechanism called desensitization. Receptor desensitization as well as receptor internalization is often mediated by arrestins. The phosphorylation of agonist-stimulated GPCRs by G protein-coupled receptor kinases (GRK) leads to the binding of arrestins [14]. Arrestins also interact with clathrin heavy chain to induce GPCR internalization via clathrin-coated pits [15]. The role of β-arrestin-1 (also called arrestin-2) in the internalization process has been argued using the β2-adrenergic receptor: overexpression of β-arrestin-1 increases receptor internalization [15] whereas dominant negative arrestin mutants that selectively bind to clathrin inhibit agonist-induced sequestration of the β2-adrenergic receptor [16]. By contrast, internalization of the muscarinic m2 receptor has been shown to be arrestin-independent although the receptor is phosphorylated by GRKs, binds arrestins and desensitizes [17]. Interestingly, GPCRs were also able to bind the adapter protein AP2 that could serve as a substitute for β-arrestin in clathrin-mediated endocytosis [18]. This raises the question of the exact relationships between internalization and desensitization for which arrestins may play either a common or a dissociated role. Recent studies have shown that sst2A efficiently desensitizes upon SRIF treatment [19] and that the desensitization mechanism involves interaction with arrestins [20]. However, a direct involvement of β-arrestin-1 in sst2A internalization or desensitization was not established. In this work, we demonstrate that the interaction of β-arrestin-1 with the sst2A receptor expressed in CHO cells desensitizes the receptor-induced decrease of cAMP level without affecting its ability to internalize.

2. Materials and methods

2.1. Materials

Somatostatin-14 (SRIF) and Tyr^0-[D-Trp^8]-SRIF were from Peninsula Laboratories. Tyr^0-[D-Trp^8]-SRIF was iodinated and purified as described previously [3]. pcDNA3 plasmids containing the β-arrestin-1 or the deleted β-arrestin-1 (319–418) (Δ-arrestin-1) sequences were generous gifts from Dr. Jeffrey L. Benovic (Philadelphia, PA, USA). Ham F12 medium, geneticin and gentamicin were purchased from Life Technologies; fetal calf serum was from Biowest; polyclonal rabbit anti-c-myc and goat anti-β-arrestin-1 antibodies were from Santa Cruz Biotechnology; FITC donkey anti-rabbit and Texas red donkey anti-goat antibodies were from Molecular Probes. Forskolin, isobutylmethylxanthine and pertussis toxin were from Sigma France; transfection reagent DAC 30 was from Eurogentec, Seraing, Belgium.

2.2. Cell culture and transfection

CHO cells were grown in Ham F12 medium with 10% fetal calf serum supplemented with gentamicin (50 μg/ml). Cells were stably transfected with the cDNA of the mouse sst2A receptor bearing a N-terminal c-myc epitope in pcDNA3 (Invitrogen) using DAC 30. Following selection with geneticin, a clone expressing 600 fmol/mg of sst2A receptor was used for further experiments. Transient transfections of β-arrestin-1 or Δ-arrestin-1 were performed using the DEAE-dextran precipitation method [21]. The percentage of cells that overexpress β-arrestin-1 or Δ-arrestin-1 was calculated to be 20–30% from confocal microscopic analysis. Membranes from non-

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Abbreviations: SRIF, somatotropin release inhibitory factor; sst2A, SRIF receptor type 2A; GPCR, G protein-coupled receptor; PheAsO, phenyl arsine oxide

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transfected CHO cells were totally devoid of specific [^{125}I]Tyr^0-[D-Trp^8]-SRIF binding.

2.3. Internalization experiments

Internalization experiments were performed on cells plated in 12-mm well culture dishes as previously described [3]. Briefly, cells were preincubated at 37°C in the absence of drugs or with sucrose (0.45 M) in an Earle's Tris–HEPES buffer for 30 min. Cells were then incubated with 0.3 nM [^{125}I]Tyr^0-[D-Trp^8]-SRIF for various times and washed twice with 0.5 ml of equilibration buffer, then twice with 0.5 ml of an acid–NaCl buffer (Earle's buffer titrated to pH 4 with acetic acid and in which 0.5 M NaCl has been added) for 2 min to remove non-sequestered radioactivity (acid–NaCl wash). Cells were harvested with 1 ml of 0.1 N NaOH and counted in a γ-counter. Non-specific binding was determined in the presence of 1 μM unlabeled SS14.

2.4. Recycling experiments

Cells were first incubated with 100 nM unlabeled SRIF for 15 min at 37°C to induce internalization. The peptide remaining on the cell surface was removed by a series of ice-cold washes: three washes with Earle's Tris–HEPES buffer, two washes with 150 mM NaCl, 5 mM acetic acid, and three more washes with Earle's Tris–HEPES buffer. Fresh Earle's Tris–HEPES buffer was added and cells were incubated at 37°C for various times. In some cases, the medium was removed and replaced by fresh medium every 5 min. The amount of cell surface receptor was then measured in binding assays carried out with 0.5 nM [^{125}I]Tyr^0-[D-Trp^8]-SRIF for 30 min either at 37°C in the presence of the internalization blocker phenyl arsine oxide (PheAsO) or at 4°C without PheAsO. The non-specific binding was determined in the presence of 1 μM unlabeled SS14.

2.5. Western blot analysis

To control the expression level of β-arrestin-1 or Δ-arrestin-1 in transfected cells, proteins from cell homogenates were separated on a 12% polyacrylamide gel according to Laemmli [22], and transferred on nitrocellulose membranes (0.2 μm). Immunoblotting was performed as previously described [23], using a 1:500 dilution of the goat anti-β-arrestin-1 antibody which recognizes the C-terminus of the protein, and revealed by the Lumilight enhanced chemiluminescence method (Roche Diagnostics).

2.6. Confocal microscopy experiments

CHO cells expressing the sst2A were transfected either with β-arrestin-1 or with the deleted β-arrestin-1 (319–418) (Δ-arrestin-1) and grown on 12-mm coverslips. After preincubation for 10 min at 37°C in Earle's Tris–HEPES buffer, cells were incubated in the presence or in the absence of 10 nM SRIF at 37°C for 5 min. After a rapid washing step, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min at room temperature, washed twice in PBS, then incubated with 50 mM NH_4Cl in PBS for 10 min to quench any excess of free aldehyde groups. Fixed cells were permeabilized by incubation in PBS containing 10% horse serum and 0.05% Triton X-100 for 20 min at room temperature, then incubated with 1:500 dilution for the goat C-terminal anti-β-arrestin-1 polyclonal antibody and a 1:250 dilution of a rabbit anti-c-myc antibody in PBS buffer containing 5% horse serum and 0.05% Triton X-100 for 1 h. Cells were rinsed three times in PBS buffer and incubated with a FITC-conjugated donkey anti-rabbit antibody and a Texas red-conjugated donkey anti-goat antibody diluted 1:600 in PBS containing 5% horse serum and 0.05% Triton X-100 for 45 min. After two washes with PBS and one wash with water, coverslips were mounted on glass slides with Mowiol for confocal microscopy examination. Labeled cells were visualized under a Leica laser scanning confocal microscope (TCS-SP) equipped with a DM-IRBE inverted microscope and an argon–krypton laser. Samples were scanned under both 488 nm and 647 nm excitation wavelengths. Images were acquired as single transcellular optical sections and averaged over 16 scans/frame.

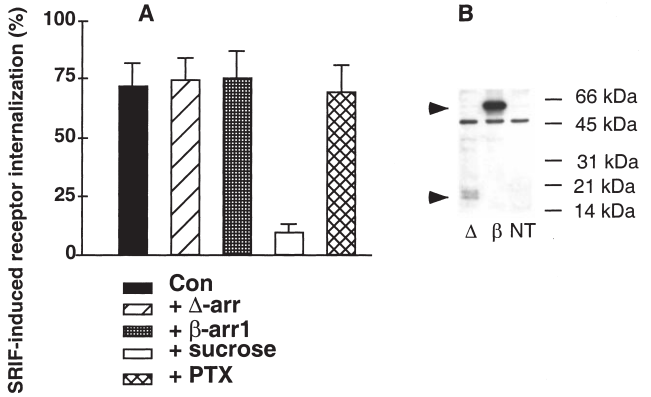

Fig. 1. Effects of sucrose, pertussis toxin and β-arrestin-1 or Δ-arrestin-1 overexpression on the level of sst2A internalized into stable c-myc-tagged sst2A transfected-CHO-cells. A: In the control experiment (CON) the amount of SRIF internalized into sst2A-transfected CHO cells was measured after 30 min. The effects of sucrose, pertussis toxin (PTX), and β-arrestin-1 (β-arr1) or Δ-arrestin-1 (Δ-arr) cotransfection are shown. Data represent the means ± S.E.M. of three to five independent experiments. B: Western blot analysis of the overexpression of β-arrestin-1 (lane 2, β) and Δ-arrestin-1 (lane 1, Δ) as compared to non-transfected CHO cells (lane 3, NT). Top arrowhead indicates β-arrestin-1 overexpression, bottom arrowhead shows the expression of Δ-arrestin-1.

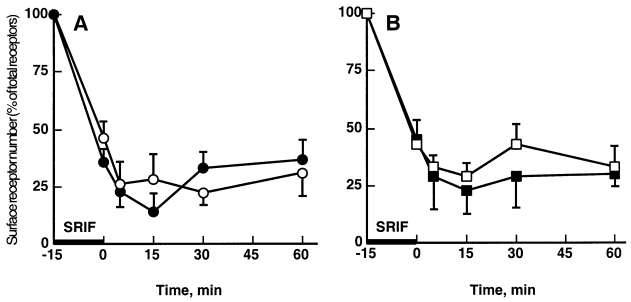

Fig. 2. Time course of c-myc-tagged sst2A receptor recycling. A: After induction of receptor sequestration for 15 min with 100 nM SRIF, the peptide was removed by acid washes and cells were incubated either with fresh medium for indicated times at 37°C (closed circles) or with fresh medium changed every 5 min up to indicated times at 37°C (open circles). The amount of cell surface receptor was then determined as described in Section 2. B: The same experiments carried out on cells cotransfected with β-arrestin-1 (closed squares) or with Δ-arrestin-1 (open squares). Data are means ± S.E.M. from at least two different experiments.

2.7. Measurement of cAMP content in CHO-sst2A cells

Cells grown in 35-mm dishes were incubated in complete Ham F12 medium with [^{3}H]adenine (1 μCi/ml) for 18 h in the presence or in the absence of pertussis toxin (100 ng/ml). Cells were preincubated in the Earle's Tris–HEPES buffer containing 1% bovine serum albumin and 0.2 mM isobutylmethylxanthine for 30 min at 37°C, then incubated for 30 min in the same buffer containing forskolin (10 μM) and increasing concentrations of SRIF. The reaction was stopped by removing the medium and adding 500 μl of ice-cold 5% trichloroacetic acid, 2 mM ATP and 2 mM cAMP for 15 min at 4°C.

For desensitization experiments, the preincubation step was performed in the presence of 100 nM SRIF, followed by two acid washes for 2 min and one wash with the incubation buffer before incubation with forskolin and SRIF. The tritiated cAMP content was measured by chromatography on Dowex 50W-X8 columns as previously described [24].

3. Results

3.1. Internalization properties of the sst2A in CHO cells

In order to determine the role of β-arrestin-1 on the internalization of [^{125}I]Tyr^0-[D-Trp^8]-SRIF in CHO cells expressing the N-terminal c-myc-tagged sst2A receptor, we measured the amount of iodinated ligand sequestered at 37°C on whole cells under various experimental conditions. Preliminary experi-

ments have shown that the binding affinity of the c-myc-tagged sst2A receptor for [^{125}I]Tyr^0-[D-Trp^8]-SRIF (0.3 nM) was identical to that of the wild type receptor expressed in CHO cells. Measurement of the radioactivity associated with cells stably transfected with the sst2A after acid–NaCl wash revealed that 72% of total [^{125}I]Tyr^0-[D-Trp^8]-SRIF bound after 40 min was sequestered (Fig. 1A). The internalization process was efficiently inhibited by hyperosmolar sucrose (Fig. 1A). Further transfection of these cells with either β-arrestin-1 or its dominant negative Δ-arrestin-1 did not modify the amount of internalized ligand (Fig. 1A). Pretreatment of cells with pertussis toxin was without effect on the level of sst2A internalization (Fig. 1A). Overexpression of both β-arrestin-1 and its dominant negative Δ-arrestin-1 was verified by Western blotting of transfected cell extracts (Fig. 1B). The percentage of cells transfected with β-arrestin-1 constructs was calculated to be approximately 20–30% in all cases.

3.2. Receptor recycling

We wanted to know whether the sst2A receptor was able to recycle to the cell surface after internalization. Measurement of reappearance of the receptor to the plasma membrane after activation of the sequestration process with SRIF clearly showed that most of the internalized receptors remained intracellular up to 60 min (Fig. 2A). Washing out the medium every 5 min during the recycling incubation did not modify the absence of recycling of the sst2A receptor (Fig. 2A). This result indicates that the sst2A does not recycle efficiently and rapidly to the plasma membrane. When experiments were

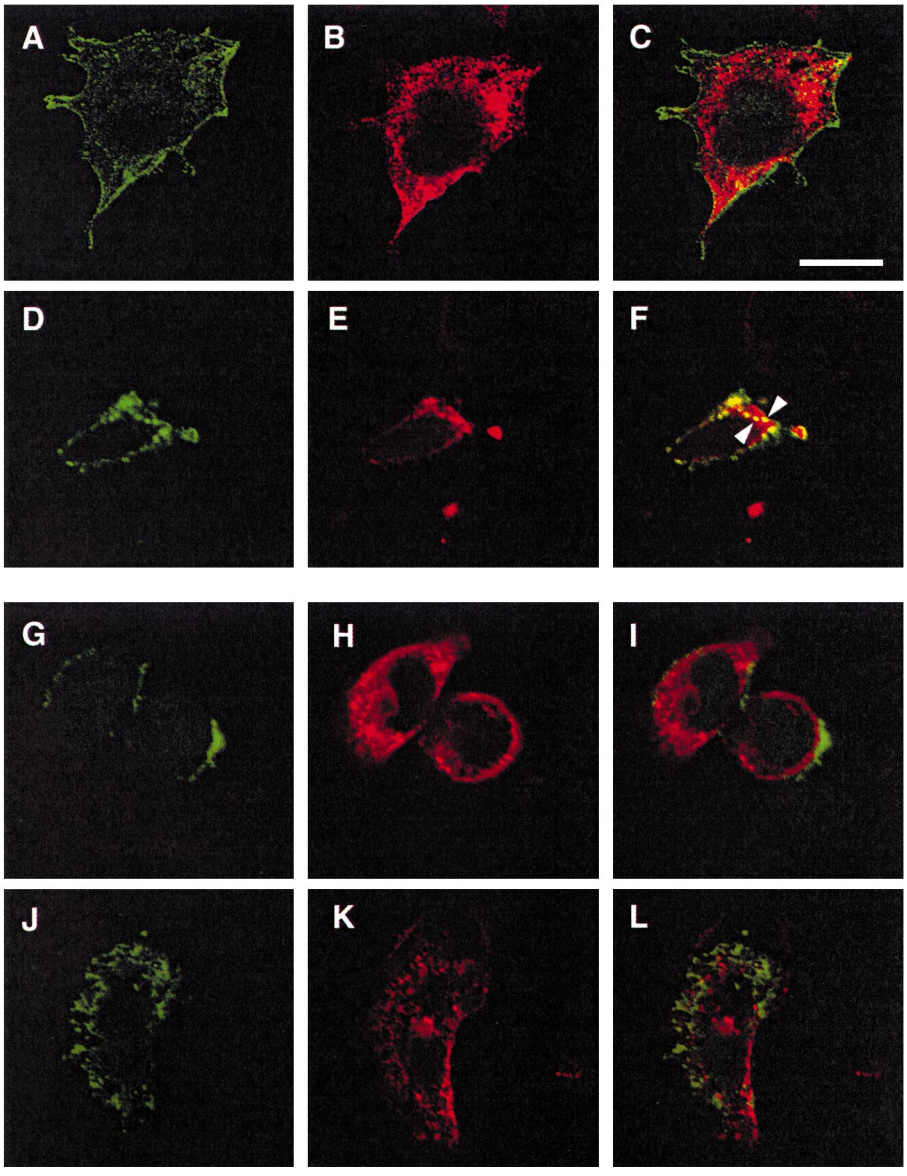

Fig. 3. Immunolocalization of c-myc-tagged sst2A and β-arrestin-1 in transfected CHO-sst2A cells. CHO cells expressing c-myc-tagged sst2A were transiently transfected with β-arrestin-1 or Δ-arrestin-1 and either immediately fixed (A–C and G–I) or treated with 100 nM SRIF at 37°C for 5 min prior to fixation (D–F and J–L). Anti-c-myc FITC labeling (A, D, G, J) and labeling of anti-C-terminal β-arrestin-1 which recognize both β-arrestin-1 (B and E) and Δ-arrestin-1 (H and K) revealed with Texas red-conjugated donkey anti-goat antibody are illustrated. Overlays of FITC-c-myc-sst2A and β-arrestin-1 fluorescent labeling are shown in C and F. The yellow color indicates colabeling. Arrowheads show clusters of colabeling. Overlays of FITC-c-myc-sst2A and Δ-arrestin-1 fluorescent labeling are shown in I and L. Scale bar: 10 μm.

carried out on CHO cells co-transfected with either β-arrestin-1 or the dominant negative Δ-arrestin-1, no more recycling of internalized sst2A receptor was observed (Fig. 2B). We also checked that the amount of [^{125}I]Tyr^0-[D-Trp^8]-SRIF released in the medium during the recycling incubation never exceeded 10% of the total amount of sequestered radioactive ligand (not shown). This amount of recycled ligand is much too low to induce a significant modification of the cell surface receptor.

3.3. Colocalization of sst2A and β-arrestin-1

Fluorescence immunolabeling of β-arrestin-1 and sst2A in cotransfected CHO-cells is shown in Fig. 3. In the absence of SRIF, the receptor was essentially localized at the cell surface (Fig. 3A). Following 5 min of incubation with SRIF, sst2A receptor immunolabeling was clearly translocated from the cell surface to intracellular endosomal compartments (Fig. 3D). In the absence of peptide, the labeling obtained in cells overexpressing β-arrestin-1 was diffuse throughout the cytoplasm (Fig. 3B) and remained intracellular following SRIF activation (Fig. 3E). Merge imaging of both labels clearly showed a colocalization of the receptor with β-arrestin-1 in some intracellular endosomal vesicles only after stimulation with SRIF (Fig. 3F). In cells overexpressing Δ-arrestin-1, no colocalization was observed with the sst2A receptor (Fig. 3I,L) whatever the conditions, although the SRIF-induced internalization of the c-myc-tagged receptor in endosomal compartments was clearly maintained (Fig. 3J).

3.4. Desensitization of sst2A receptor, role of β-arrestin-1

The ability of SRIF to inhibit the forskolin-induced increase of cAMP level was first measured in CHO cells expressing the sst2A with or without preincubation with 100 nM of peptide. SRIF inhibited dose-dependently the forskolin-stimulated cAMP level with a maximal effect (45% inhibition) at 100 nM (Fig. 4A). Transfection of either β-arrestin-1 or Δ-arrestin-1 did not modify SRIF inhibition of cAMP level (not shown). When cells were pretreated for 30 min in the presence of 100 nM SRIF and washed, forskolin remained able to stimulate cAMP formation but SRIF could no longer decrease the forskolin-stimulated levels of cAMP. This result demonstrates that the coupling of sst2A to adenylate cyclase

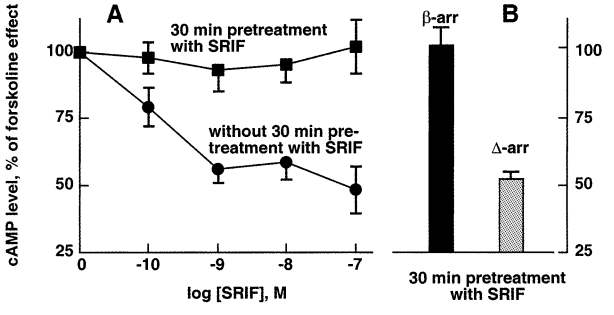

Fig. 4. Effects of β-arrestin-1 and Δ-arrestin-1 on SRIF-induced activation and desensitization in CHO-c-myc-sst2A cells. A: Concentration-dependent inhibition of forskolin (100 μM)-stimulated cAMP level in CHO-sst2A cells by SRIF with 30 min pretreatment of cells (squares) or without 30 min pretreatment of cells (circles) with 100 nM SRIF. B: Effects of β-arrestin-1 (β-arr) or Δ-arrestin-1 (Δ-arr) transfection on SRIF-induced desensitization in CHO-sst2A cells. Data are means ± S.E.M. from at least three independent experiments.

was desensitized by preincubation with the peptide. In CHO-sst2A cells transfected with β-arrestin-1, the receptor desensitization was maintained, since no SRIF inhibitory effect was observed after preincubation of transfected cells with the peptide (Fig. 4B). By contrast, in CHO-sst2A cells transfected with Δ-arrestin-1, SRIF kept its ability to inhibit forskolin stimulation of cAMP even after pretreatment with 100 nM SRIF (Fig. 4B), indicating that desensitization of sst2A by SRIF was prevented by Δ-arrestin-1.

4. Discussion

The present study confirms the existence of an interaction between β-arrestin-1 and the sst2A receptor after activation by SRIF and demonstrates that this interaction is crucial for desensitization but not for internalization of the receptor.

Evidence for arrestin involvement on agonist-mediated desensitization of endogenous SRIF receptors has been previously shown in HEK293 cells in which reduction of arrestin levels attenuated desensitization of SRIF receptor [20]. However, in HEK293 cells, the subtype of SRIF receptor that was regulated by arrestins had not been identified [20], although it had been reported earlier that the sst2A receptor was expressed at low levels in HEK cells [25]. Here, we show that SRIF activation induces a cellular translocation of β-arrestin-1 from a diffuse cytoplasmic location to vesicular endosomal compartments also containing the sst2A receptor. Previous studies have indicated that arrestins can traffic with some internalized GPCRs into early endosomes [26,27]. However, in the case of sst2A receptor, the association with β-arrestin-1 is not essential since we demonstrate here that overexpression of either β-arrestin-1 or Δ-arrestin-1 does not affect receptor internalization. An arrestin-independent internalization process has already been described for the m1, m3 and m4 subtypes of muscarinic cholinergic receptors [28]. By contrast, the β-arrestin-1-sst2A interaction appears crucial for receptor desensitization, since transfection of Δ-arrestin-1 which disrupts the interaction between endogenous arrestins and clathrin totally abolishes sst2A desensitization.

SRIF-induced sst2B receptor desensitization has been suggested to be dependent on receptor internalization in the neuroblastoma × glioma hybrid cell line NG108-15, although this process was shown to be independent of the GRKs [29]. All these data suggest that the interaction of β-arrestin with a GPCR upon ligand activation is observed in almost all cases but that this interaction may be either passive (i.e. for muscarinic m1 and sst2A) or active (i.e. for β2-adrenergic) towards the receptor internalization process. By contrast, even if this interaction is ineffective on receptor internalization, it may contribute to desensitization. Although a prolonged β-arrestin–GPCR interaction has been shown to be responsible for the absence of rapid receptor recycling for the vasopressin V2 receptor [30], this is not the case for the sst2A receptor. Even in the absence of interaction with arrestin, i.e. in the presence of Δ-arrestin-1, no recycling of receptor was observed (Fig. 2B). The inability of the sst2A to rapidly recycle in AtT20 cells was already observed by fluorescence immunocytochemistry. After prolonged stimulation of AtT20 cells with SRIF, the labeling of the receptor was concentrated in the cytoplasmic core next to the nucleus, whereas the cell surface immunolabeling was almost totally lost [24]. The fact that the receptor has lost its ability to be desensitized in the

presence of the dominant negative Δ-arrestin-1 although a large proportion of receptor was sequestered suggests that the desensitization induced by β-arrestin-1 binding mainly involves cell surface sst2A receptor. This also indicates that the two processes are dissociated and that internalization is not a prerequisite for desensitization. In the same way, recycling and desensitization are likely not strictly interconnected for the sst2A receptors. Although β-arrestin-1 was colocalized with internalized receptors and was described to bind receptors involved in the desensitization, this interaction is certainly no longer essential for receptor sequestration for which β-arrestin-1 could be replaced by other component(s) of the endocytotic machinery. By contrast, this interaction is crucial for homologous desensitization of sst2A receptors.

In conclusion, we have shown that β-arrestin-1 interacts with the mouse somatostatin receptor subtype sst2A and that this interaction is a necessary prerequisite for receptor desensitization. By contrast, the β-arrestin-1-sst2A interaction is not essential for intracellular sequestration of the SRIF–receptor complex. Thus, different intracellular protein partners are involved in the mechanisms of internalization and desensitization of GPCRs.

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References

[1] Hoyer, D., Bell, G.I., Berelowitz, M., Epelbaum, J., Feniuk, W., Humphrey, P.P.A., O'Carroll, A.M., Patel, Y.C., Schonbrunn, A., Taylor, J.E. and Reisine, T. (1995) Trends Pharmacol. Sci. 16, 86–88.
[2] Reisine, T. and Bell, G. (1995) Endocr. Rev. 16, 427–442.
[3] Nouel, D., Gaudriault, G., Houle, M., Reisine, T., Vincent, J.P., Mazella, J. and Beaudet, A. (1997) Endocrinology 138, 296–306.
[4] Hukovic, N., Panetta, R., Kumar, U. and Patel, Y.C. (1996) Endocrinology 137, 4046–4049.
[5] Koch, B.D., Dorflinger, L.J. and Schonbrunn, A. (1985) J. Biol. Chem. 260, 13138–13145.
[6] Koch, B.D., Blalock, J.B. and Schonbrunn, A. (1988) J. Biol. Chem. 263, 216–225.
[7] Yatani, A., Codina, J., Sekura, R.D., Birnbaumer, L. and Brown, A.M. (1987) Mol. Endocrinol. 1, 283–289.
[8] White, R.E., Schonbrunn, A. and Armstrong, D.L. (1991) Nature 351, 570–573.
[9] Bito, H., Moro, M., Sakanaka, C., Takano, T., Honda, Z., Gotoh, Y., Nishida, E. and Shimizu, T. (1994) J. Biol. Chem. 269, 12722–12730.
[10] Duerson, K., White, R.E., Jiang, F., Schonbrunn, A. and Armstrong, D.L. (1996) Neuropharmacology 35, 949–961.
[11] Florio, T., Rim, C., Hershberger, R.E., Loda, M. and Stork, P.J.S. (1994) Mol. Endocrinol. 8, 1289–1297.
[12] Tomura, H., Okajima, F., Akbar, M., Abdul Majid, M., Sho, K. and Kondo, Y. (1994) Biochem. Biophys. Res. Commun. 200, 986–992.
[13] Hou, C., Gilbert, R.L. and Barber, D.L. (1994) J. Biol. Chem. 269, 10357–10362.
[14] Carman, C. and Benovic, J.L. (1988) Curr. Opin. Neurobiol. 8, 335–344.
[15] Goodman Jr., O.B., Krupnick, J.G., Santini, F., Gurevich, V.V., Penn, R.B., Gagnon, A.W., Keen, J.H. and Benovic, J.L. (1996) Nature 383, 447–450.
[16] Krupnick, J.G., Santini, F., Gagnon, A.W., Keen, J.H. and Benovic, J.L. (1997) J. Biol. Chem. 272, 32507–32512.
[17] Pals-Rylaarsdam, R., Gurevich, V.V., Lee, K.B., Ptasienski, J.A., Benovic, J.L. and Hosey, M.M. (1997) J. Biol. Chem. 272, 23682–23689.
[18] Laporte, S.A., Oakley, R.H., Zhang, J., Holt, J.A., Ferguson, S.S.G., Caron, M.G. and Barak, L.S. (1999) Proc. Natl. Acad. Sci. USA 96, 3712–3717.
[19] Hipkin, R.W., Friedman, J., Clark, R.B., Eppler, C.M. and Schonbrunn, A. (1997) J. Biol. Chem. 272, 13868–13876.
[20] Mundell, S.J. and Benovic, J.L. (2000) J. Biol. Chem. 275, 12900–12908.
[21] Cullen, B.R. (1987) Methods Enzymol. 152, 684–695.
[22] Laemmli, U.K. (1970) Nature 227, 680–685.
[23] Navarro, V., Martin, S., Sarret, P., Nielsen, M.S., Petersen, C.M., Vincent, J.P. and Mazella, J. (2001) FEBS Lett. 495, 100–105.
[24] Sarret, P., Nouel, D., Dal Farra, C., Vincent, J.P., Beaudet, A. and Mazella, J. (1999) J. Biol. Chem. 274, 19294–19300.
[25] Law, S.F., Yasuda, K., Bell, G.I. and Reisine, T. (1993) J. Biol. Chem. 268, 10720–10727.
[26] McConalogue, K., Dery, O., Lovett, M., Wong, H., Walsh, J.H., Grady, E.F. and Bunnett, N.W. (1999) J. Biol. Chem. 274, 16257–16268.
[27] Zhang, J., Bark, L.S., Anborgh, P.H., Laporte, S.A., Caron, M.G. and Ferguson, S.S.G. (1999) J. Biol. Chem. 274, 10999–11006.
[28] Lee, K.B., Pals-Rylaarsdam, R., Benovic, J.L. and Hosey, M.M. (1998) J. Biol. Chem. 273, 12967–12972.
[29] Beaumont, V., Hepworth, M.B., Luty, J.S., Kelly, E. and Henderson, G. (1998) J. Biol. Chem. 273, 33174–33183.
[30] Oakley, R.H., Laporte, S.A., Holt, J.A., Barak, L.S. and Caron, M.G. (1999) J. Biol. Chem. 274, 32248–32257.