Role of the Proline-rich Domain of Dynamin-2 and Its Interactions with Src Homology 3 Domains during Endocytosis of the AT₁ Angiotensin Receptor*

Márta Szaszáz, Zsuzsanna Gáborik, Gábor Turu, Peter S. McPherson‡, Adrian J. L. Clark§, Kevin J. Catt’s, and László Hunyady¶

From the Department of Physiology, Semmelweis University, Faculty of Medicine, H-1088 Budapest, Hungary, the Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec H3A 2B4, Canada, the Department of Endocrinology, Barts & the London, Queen Mary, University of London, London EC1A 7BE, United Kingdom, and NICHD, National Institutes of Health, Bethesda, Maryland 20892-4510

In nonneural tissues, the dynamin-2 isoform participates in the formation of clathrin-coated vesicles during receptor endocytosis. In this study, the mechanism of dynamin-2 action was explored during endocytosis of the G protein-coupled AT₁A angiotensin receptor expressed in Chinese hamster ovary cells. Dynamin-2 molecules with mutant pleckstrin homology domains or deleted proline-rich domains (PRD) exerted dominant negative inhibition on the endocytosis of radiolabeled angiotensin II. However, only the PRD mutation interfered with the localization of the dynamin-2 molecule to clathrin-coated pits and reduced the inhibitory effect of the GTPase-deficient K44A mutant dynamin-2. Green fluorescent protein-tagged Src homology 3 (SH3) domains of endophilin I and amphiphysin II, two major binding partners of dynamins, also inhibited AT₁A receptor-mediated endocytosis of angiotensin II. These effects were partially or fully, respectively, restored by the overexpression of dynamin-2. Transient overexpression of these SH3 domains also reduced the localization of dynamin-2 to clathrin-coated pits. These data indicate that similar to the recruitment of dynamin-1 during the recycling of synaptic vesicles, interaction of the dynamin-2 PRD with SH3 domains of proteins such as the amphiphysins and endophilins is essential for AT₁A receptor endocytosis. This mechanism could be of general importance in dynamin-dependent endocytosis of other G protein-coupled receptors in nonneural tissues.

The AT₁ angiotensin receptor (AT₁-R) is a member of the GPCR superfamily and mediates the physiological actions of the octapeptide hormone, Ang II, on cardiovascular regulation and salt/water balance. In rats and mice, the AT₁-R has functionally very similar AT₁A and AT₁B subtypes, which have greater than 90% sequence identity but different tissue distributions. Binding of Ang II to AT₁-R initiates conformational changes that lead to activation of their cognate G protein(s), predominantly Gq, in numerous Ang II target tissues. The receptors utilize a number of signaling pathways including stimulation of phospholipase C, causing Ca²⁺ signal generation and activation of protein kinase C isoforms and small G proteins, stimulation of receptor and nonreceptor tyrosine kinases, and activation of mitogen-activated protein kinases (1–3). In parallel with these signaling events, Ang II also causes rapid internalization of the AT₁-R (4, 5). Initial studies performed at saturating Ang II concentrations suggested that dynamin-independent mechanisms may mediate AT₁-R internalization (6). However, at physiological hormone concentrations the major mechanism of AT₁-R internalization is dynamin- and β-arrestin-dependent endocytosis via clathrin-coated vesicles (7–10).

Clathrin coat formation is thought to begin with recruitment of the AP2 clathrin adaptor complex to the plasma membrane, where it serves as a template for the assembly of the clathrin lattice. Studies on recycling of synaptic vesicles and internalization of nutrient and growth factor receptors have demonstrated that invagination of clathrin-coated pits and fission of clathrin-coated vesicles requires the recruitment of several accessory proteins, including dynamin, amphiphysin, endophilin, and synaptojanin (11–13). Dynamin is a 100-kDa GTPase that is thought to act at the fission step and is present in the cytosol as dimers or tetramers (14). During receptor endocytosis, dynamin assembles into ringlike structures around the neck of budding clathrin-coated pits, where it functions directly or indirectly in pinching off vesicles from the plasma membrane (15). Dynamin alone can polymerize into rings and can evaginate spherical liposomes into narrow tubules surrounded by polymerized dynamin (13, 16–19). However, other molecules interact with dynamin in vivo to facilitate its function and to assist in its recruitment to clathrin-coated pits (11, 12, 20).

Dynamin has several functional domains including a N-terminal GTPase domain, a PH domain, and a C-terminal proline-rich domain (PRD) (13, 15). The GTPase activity of dynamin is essential for its function, and GTPase-deficient mutants of dynamin act as dominant negative inhibitors of endocytosis (13, 15). The PH domain is a structural motif that is found in hundreds of proteins and has been shown to bind

SH3-Endo, SH3 domain of endophilin I; SH3-Nck, N-terminal SH3 domain of Nck; GST, glutathione S-transferase.

Received for publication, January 24, 2002
Published, JBC Papers in Press, March 29, 2002, DOI 10.1074/jbc.M200778200

* This work was supported in part by Wellcome Trust Collaborative Research Initiative Grant 051804/Z/97/Z, Hungarian Ministry of Education Grants FKFP-0318/1999 and OMFB 02489/2000, Hungarian Science Foundation Grant OTKA T-032179, Hungarian Ministry of Health Grant ETT 315/2000, and Canadian Institutes of Health Research Operating Grant MT-1346 (endophilin grant). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed: Dept. of Physiology, Semmelweis University, Faculty of Medicine, H-1444 Budapest, 8. P.O. Box 259, Hungary, Tel.: 36-1-266-2755 (ext. 4041); Fax: 36-1-266-6504; E-mail: Hunyady@puskin.sote.hu.

§The abbreviations used are: AT₁-R, type 1 angiotensin receptor; GPCR, G protein-coupled receptor; Ang II, angiotensin II; CHO, Chinese hamster ovary; dyn1, dynamin-1; dyn2, dynamin-2; GFP, green fluorescent protein; GPCR, G protein-coupled receptor; HA, influenza hemagglutinin; PH, pleckstrin homology; PRD, proline-rich domain; SH3, Src homology 3; SH3-Amp, SH3 domain of amphiphysin II;
with different preferences to various phosphoinositides. Certain PH domains that bind with high affinity and high specificity for phosphoinositides are capable of driving membrane recruitment. The PH domain of dynamin binds phosphoinositides with low affinity, and oligomerization of the molecule is required for strong binding of dynamin to inositol lipid-containing membranes in vitro. The importance of the PH domain in dynamin function is indicated by the ability of PH domain mutant dynamins with impaired phosphoinositide binding to act as dominant negative inhibitors of receptor endocytosis.

The PRD of dynamin interacts in vitro with many SH3 domain-containing proteins, including proteins with established roles in endocytosis and recycling of synaptic vesicles. These endocytic proteins include amphiphysin and endophilin, which has been implicated in the recruitment of dynamin to clathrin-coated vesicles. However, endophilin also stimulates membrane vesiculation independent of its enzymatic activity. Although the major binding partner of endophilin is synaptojanin, a phosphotyrosinol 5-phosphatase, it also binds to the PRD of dynamin. Previous studies have suggested that dynamin can be targeted to the membrane by interaction with SH3 domain-containing proteins.

Most of the data on dynamin's interaction with SH3 domain-containing proteins, such as amphiphysin and endophilin, was obtained using dyn1, the neuronal isoform of the molecule. These interactions are required for the recycling of synaptic vesicles via a clathrin-mediated process, and a similar process may operate during the endocytosis of hormone receptors in nonneuronal tissues. It is noteworthy that most endocytic proteins have neural and nonneuronal isoforms with very similar domain structures. In addition to its role in the formation of endocytic vesicles at the cell surface, dyn2, the ubiquitous isoform of dynamin, has been implicated in the invagination of coated vesicles. However, endophilin also stimulates membrane vesiculation independent of this enzymatic activity. Although the major binding partner of endophilin is synaptojanin, a phosphotyrosinol 5-phosphatase, it also binds to the PRD of dynamin. Previous studies have suggested that dynamin can be targeted to the membrane by interaction with SH3 domain-containing proteins.

The role of dynamin during endocytosis of many GPCRs has been well established using GTPase-deficient dynamin mutants. However, little is known about its mechanism of action in this process. In some GPCRs, including the AT1α-R, PH domain mutant dynamin has a dominant negative inhibitory effect on agonist-induced receptor endocytosis. Endophilins directly interact with the β2-adrenergic receptor and stimulate its agonist-induced internalization, but the role of their association with dynamin has not been demonstrated during the endocytosis of GPCRs. The role of the PRD in recruitment of dyn2 to clathrin-coated pits in nonneuronal tissues has also not been elucidated. The present study was performed to determine whether the role of ubiquitously expressed dyn2 isoform during agonist-induced endocytosis of the G protein-coupled AT1α receptor in nonneuronal cells is analogous to that of the neuronal isoform of dynamin, dyn1, during the recycling of synaptic vesicles and endocytosis of nutrient and growth factor receptors.
Dynamin-2 and SH3 Interactions in AT₁ Receptor Endocytosis

RESULTS

Dominant Negative Inhibition of AT₁-R Endocytosis by PRD and PH Domain Mutants of dyn2—To study the role of the PRD of dyn2 during AT₁-R endocytosis, a dyn2 mutant was created (dyn2-ΔPRD), in which the entire C-terminal proline-rich domain was deleted commencing at proline 746 (Fig. 1A). When co-expressed with the AT₁A-R in CHO cells, this construct had a dominant negative inhibitory effect on the endocytosis of the receptor (Fig. 2A). The expression levels of the mutant dyn2 constructs in CHO cells are shown in Fig 1B. However, the extent of inhibition was smaller than that caused by the GTPase-deficient mutant, dyn2-K44A. As previously shown (40), dyn2-K44A transformed co-localization of dyn2 with 0.5 μg of AT₁A-R cDNA and 0.5 μg of wild-type dyn2 (●), dyn2-ΔPRD (▲), dyn2-K44A (■), and dyn2-K44A/ΔPRD (○). Endocytosis of 125I-Ang II was measured as described under “Experimental Procedures.” Data are shown as means ± S.E. of four independent experiments, each performed in duplicate. B, endocytosis of 125I-Ang II in CHO cells co-transfected with 0.5 μg of AT₁A-R cDNA and 0.5 μg of wild-type dyn2 (●), dyn2-K535A (●), dyn2-K44A (■), or dyn2-K44A/K535A (○). Experimental conditions were the same as in A.

shown to exert dominant negative inhibitory effects on AT₁-R endocytosis (7, 52). Combination of the K535A and the K44A mutations increased the dominant negative inhibition of AT₁-R endocytosis compared with the individual dyn2-K535A mutation, and its effect was similar to that of the dyn2-K44A mutation (Fig. 2B). These data suggest that, unlike the PRD, the PH domain of dyn2 is not required for proper localization of the molecule during AT₁-R endocytosis.

Localization of the Mutant Forms of dyn2—The intracellular localization of transiently expressed dynamins in CHO cells was analyzed by immunostaining to detect the HA epitope tag attached to the wild-type and mutant dyn2 (40). Wild-type dyn2 showed a diffuse cytoplasmic pattern and also localized to the plasma membrane (Fig. 3A). Replacement of lysine 535 by alanine in the PH domain had no major effect on the localization of the mutant molecule as compared with wild-type dyn2 (Fig. 3B). As previously shown (40), dyn2-K44A appeared in punctate cytoplasmic structures and also accumulated in larger structures associated with the cell membrane (Fig. 3D). In contrast, whereas the dyn2-ΔPRD mutant also appeared in punctate cytoplasmic structures, no membrane localization was observed (Fig. 3C). The localization of the dyn2-K44A/K535A (Fig. 3E) and dyn2-K44A/ΔPRD (Fig. 3F) double mutant constructs was not distinguishable from that of the dyn2-K44A and the dyn2-ΔPRD, respectively.

The presence of wild-type and mutant dyn2 molecules in clathrin-coated pits was determined based on co-localization with GFP-tagged β₂-adaptin (Fig. 4), an essential component of the AP2 clathrin adapter protein (12, 55, 56). As previously
shown, β₂-adaptin-GFP was detectable in the cytosol and was co-localized with clathrin-coated pits (55). To detect enrichment of these structures at the plasma membrane, images were taken at the surface of the cell from the layer adjacent to the coverslip (Fig. 4). At the plasma membrane, both wild-type (Fig. 4B) and K535A (Fig. 4E) mutant dyn2 showed extensive co-localization (Fig. 4, C and F) with the β₂-adaptin-GFP labeling clathrin-coated pits (Fig. 4, A and D). The K44A mutant dyn2 (Fig. 4H) likewise showed co-localization with β₂-adaptin-GFP at the cell surface (Fig. 3J) but also appeared in clathrin-independent structures, a finding consistent with its occurrence in punctate intracellular structures (Fig. 4D) as described above. The double mutant dyn2-K44A/K535A (Fig. 4L) showed a similar partial co-localization with β₂-adaptin-GFP (Fig. 4L). However, Fig. 4, O and R, show that no significant co-localization of dyn2-ΔPRD (Fig. 4N) and dyn2-K44A/ΔPRD (Fig. 4Q) was observed with β₂-adaptin-GFP (Fig. 4, M and P), suggesting that this construct is not recruited to clathrin-coated vesicles. These data suggest that the PRD is required for recruitment of dyn2 to clathrin-coated pits at the plasma membrane and that the PH domain of the molecule has no significant role in this process.

Effects of Amphiphysin and Endophilin SH3 Domains on AT₁-R Endocytosis
—

The role of the PRD of dyn2 during AT₁-R endocytosis was also investigated by studying the effects of its potential binding partners on this process. As detailed above, earlier studies have established that amphiphysin and endophilin can interact with separate regions of the PRD of dyn2 via their SH3 domains. GFP-tagged versions of the SH3-Endo and SH3-Amph were co-expressed with the AT₁-R to study the effects of these constructs on the internalization kinetics of the receptor. The expression levels of these GFP-
Procedures.

Data are shown as means ± S.E. of three independent experiments, each performed in duplicate.

CHO cells were grown in 24-well plates and co-transfected with 0.5 µg of AT1A-R cDNA-containing plasmid (●) and 0.5 µg of GFP-tagged SH3-Endo (○), GFP-tagged SH3-Amph (△), or GFP-tagged SH3-Nck (○). Endocytosis of 125I-Ang II was measured as described under "Experimental Procedures." Data are shown as means ± S.E. of seven independent experiments, each performed in duplicate.

Fig. 5. Effects of overexpressed GFP-SH3 domains of endophilin, amphiphysin, and Nck on the kinetics of AT1A-R endocytosis. CHO cells were grown in 24-well plates and co-transfected with 0.5 µg of AT1A-R cDNA-containing plasmid (●) and 0.5 µg of GFP-tagged SH3-Endo (○), GFP-tagged SH3-Amph (△), or GFP-tagged SH3-Nck (○). Endocytosis of 125I-Ang II was measured as described under "Experimental Procedures." Data are shown as means ± S.E. of seven independent experiments, each performed in duplicate.

dynamin in vitro (57), did not affect the endocytosis of the receptor. The inhibitory actions of SH3-Endo and SH3-Amph suggest that interaction of dyn2 with these proteins is required during AT1A-R endocytosis. To determine whether these SH3 domains exerted their inhibitory effect by blocking the physiological interactions of dyn2’s PRD, the effect of overexpression of dyn2 was studied. Expression of wild-type dyn2 had a minor inhibitory effect on AT1A-R endocytosis (7). However, co-expression of dyn2 with the SH3-Endo (Fig 6A) or SH3-Amph (Fig. 6B) partially or fully, respectively, rescued the inhibitory effect of these constructs on AT1A-R endocytosis. These data suggest that the interaction of the dyn2-PRD with endophilin and amphiphysin has a role in the recruitment of dynamin to clathrin-coated pits during AT1A-R internalization.

Mislocalization of dyn2 in Cells Expressing SH3-Amph and SH3-Endo—The intracellular distribution of dyn2 was markedly affected in cells expressing the SH3 domain of endophilin compared with those expressing only dyn2 (Fig. 7, A–C). The plasma membrane localization and the even cytosolic distribution were lost, and the dynamin staining became punctate and was co-localized with the GFP-tagged SH3-Endo (Fig. 7, A–C). The plasma membrane localization of dyn2 was also lost in cells

Fig. 6. Overexpression of dyn2 interferes with the inhibitory effects of endophilin I and amphiphysin II SH3 domains on AT1A-R endocytosis. A, CHO cells were grown in 24-well plates and co-transfected with 0.5 µg of AT1A-R cDNA and 0.5 µg of dyn2 (●), 0.5 µg GFP-tagged SH3-Endo (○), or 0.5 µg GFP-tagged SH3-Endo and 0.5 µg dyn2 (■). B, CHO cells were grown in 24-well plates and cotransfected with 0.5 µg of AT1A-R cDNA and 0.5 µg of dyn2 (●), 0.5 µg of SH3-Amph (△), or 0.5 µg SH3-Amph and 0.5 µg of dyn2 (▲). Endocytosis of 125I-Ang II was measured as described under "Experimental Procedures." Data are shown as means ± S.E. of three independent experiments, each performed in duplicate.

Fig. 7. Subcellular localization of co-transfected HA epitope-tagged dyn2 and the GFP-tagged SH3-Endo, SH3-Amph, and SH3-Nck. CHO cells were co-transfected with 0.5 µg of AT1A-R cDNA and 0.5 µg of HA-dyn2 in combination with either 0.5 µg of GFP-tagged SH3-Endo (A–C), 0.5 µg of GFP-tagged SH3-Amph (D–F), or 0.5 µg of GFP-tagged SH3-Nck (G–I). After stimulation with Ang II (100 nmol/liter, 10 min, 37°C), the cells were fixed and stained with monoclonal anti-HA antibody. The fluorescence of GFP-tagged SH3-Endo (A), SH3-Amph (D), and SH3-Nck (G) are shown in green. Localization of HA-dyn2 (B, E, and H) is shown in red. Overlays are shown in C, F, and I. In the top panels (A–C), a cell with no detectable expression of GFP-tagged SH3-Endo (top right corner) shows typical plasma membrane and cytoplasmic localization of dyn2, and another cell with high expression of GFP-tagged SH3-Endo (lower left corner) shows localization of SH3-Endo and dyn2 to punctate cytosolic structures with loss of dyn2 from the plasma membrane. In the middle panels (D–F), cells with no detectable expression of GFP-tagged SH3-Amph (top right) show typical localization of dyn2. In cells that express high levels of SH3-Amph, the plasma membrane localization of dyn2 is not detectable. In the lower panels (G and H), the cell in the middle expresses GFP-tagged SH3-Nck, which does not bind to dynamin, and the characteristic plasma membrane localization of dyn2 is not affected. Typical cells are shown from a representative example of three experiments with identical results.
expressing SH3-Amph (Fig. 7, D–F), but in this case the localization of dyn2 in the cytoplasm was homogenous (Fig. 7E). GFP-tagged SH3-Nck was detectable in the cytoplasm and showed homogenous localization to the plasma membrane without accumulation in clathrin-coated pits (Fig. 7G). Coexpression of GFP-SH3-Nck did not interfere with the localization of dyn2 (Fig. 7, G–I).

**DISCUSSION**

The essential role of dynamin during the formation of clathrin-coated vesicles is well established (12, 13, 15). In addition to its action in the scission of coated vesicles, dynamin has been proposed to have a role in the invagination of clathrin-coated pits (20). As with the recycling of synaptic vesicles and the endocytosis of nutrient and growth factor receptors, numerous GPCRs internalize via a dynamin-dependent mechanism (5, 50, 51, 58, 59). Most studies on this subject have examined the role of dyn1, but many GPCRs operate in nonneuronal tissues that express the ubiquitous dyn2 isoform (60). However, there are few data on the recruitment of dyn2 to clathrin-coated pits during the endocytosis of GPCRs.

In the present experiments, kinetic analysis of AT1A-R endocytosis was performed at subnanomolar Ang II concentrations, because under these conditions the internalization of the receptor is mediated predominantly by dynamin-dependent endocytosis via clathrin-coated vesicles (7). Our observations demonstrate that the interaction of the PRD of dyn2 with SH3 domain-containing proteins is required for dynamin-dependent endocytosis of the AT1A-R molecule. Deletion of the PRD of dyn2 or overexpression of the SH3 domains of endocytic proteins such as endophilin and amphiphysin interfered with the recruitment of dyn2 to clathrin-coated pits and exerted dominant negative inhibitory effects on AT1A-R endocytosis. The dominant negative inhibitory effect of dyn2-PRD is unusual, because most expressed proteins with mutations that cause mistargeting of the molecule do not interfere with the function of the normal endogenous protein. However, dynamin exists in the cytosol as tetramers (or oligomers) before clathrin coat assembly (61), and it appears that the presence of the mutant protein in these complexes interferes with the function of the normal endogenous molecule. The role of the PRD in localization of dyn2 is also consistent with the finding that deletion of the PRD reduces the inhibitory effect of dyn2-K44A on AT1A-R endocytosis to that of ΔPRD-dyn2. An earlier study found that deletion of the PRD of dyn1 does not eliminate the inhibitory effect of the K44A mutation on transferrin receptor endocytosis (38). However, the inhibitory effect of the PRD-deleted dynamin on receptor endocytosis may explain these findings.

Co-localization of amphiphysin and endophilin with dynamin at the neck of coated pits in synaptic vesicles has been shown previously (62, 63). In the present study, expression of the SH3-Amph and SH3-Endo also inhibits endocytosis of the receptor and interferes with the localization of dynamin to clathrin-coated pits. Although expression of SH3 domains may interact nonspecifically with many different processes that involve proline-rich domains, it was shown that inhibition of receptor endocytosis with SH3 domains of endocytic proteins is fairly specific (31, 39). Furthermore, in the present study, the inhibitory effects of SH3-Amph and SH3-Endo were attenuated by overexpression of dyn2, indicating that they act by blocking interactions of the PRD of endogenous dyn2. These SH3 domains also interfered with the plasma membrane localization of dyn2. Amphiphysin has been proposed to participate in the recruitment of dynamin to clathrin-coated pits, because it binds to the clathrin heavy chain, the appendage domain of α-adaptin, dynamin, and synaptojanin (12, 47). The present data suggest that peripheral variants of amphiphysin II play a similar role in nonneuronal tissues. It has been reported recently that the SH3 domain of endophilin inhibits both late stages of invagination and scission of clathrin-coated vesicles in vitro by reducing phosphoinositide levels and interfering with the recruitment of the components of clathrin-coated pits to the plasma membrane (20). Although these mechanisms may operate under our experimental conditions, the present data suggest that endophilin also has a more specific role in dynamin recruitment because overexpression of dyn2 partially interfered with the inhibitory effect of dyn2-Endo on AT1A-R endocytosis. The importance of endophilin in dynamin recruitment is underlined by the recent identification of the binding site for endophilin SH3 domains on dynamin (PXXPR), which is present in a region of dynamin that was previously found to be the major determinant of dynamin localization to clathrin-coated pits (37, 48).

As shown earlier, a PH domain mutant dyn2 (dyn2-K535A) with reduced phosphoinositide binding also had a dominant negative inhibitory effect upon endocytosis of the AT1A-R. Although the interaction of PH domains with phosphoinositides is generally believed to be crucial for the subcellular localization of the molecule (21, 64), and PH domains are widely used to map the localization of their lipid-binding partners (23), the K535A mutation did not interfere with the localization of the molecule to clathrin-coated pits. These data indicate that the PH domain of dynamin does not act as a subcellular localization signal but does have a functional role during dynamin action. These data are consistent with earlier findings that interaction of the PH domain of dynamin with lipids increases its GTPase activity (13). This mechanism could explain the additivity of the K535A mutation with the K44A mutation, since the latter change did not cause complete inhibition of the GTPase activity of the molecule (52).

In summary, the present data demonstrate that a dyn2 mutant with deletion of the PRD and the SH3 domains of amphiphysin II and endophilin I exerts dominant negative inhibitory effects on endocytosis of the G protein-coupled AT1A-R. Confocal analysis of the localization of mutant and wild-type dyn2 and the GFP-tagged SH3 domains demonstrated that this interaction, but not the intact PH domain, is required for proper localization of the dyn2 molecule in clathrin-coated pits. These findings suggest that the actions of nonneural forms of dynamin, amphiphysin, and endophilin during dynamin-dependent endocytosis of a GPCR are similar to those described for the neuronal isoforms of these proteins.

**Acknowledgments**—The excellent technical assistance of Judit Bakacskiné Racz and Katiina Supeki is greatly appreciated. We thank Drs. K. E. Bernstein, L. Buday, M. G. Caron, S. S. G. Ferguson, K. Nakayama, and T. C. Sudhof for providing plasmid DNA constructs and Dr. László Buday for constructive suggestions.

**REFERENCES**

1. De Gasparo, M., Catt, K. J., Inagami, T., Wright, J. W., and Unger, T. (2000) Pharmacol. Rev. 52, 415–472
2. Eguchi, S., and Inagami, T. (2000) Regul. Pept. 91, 13–20
3. Berk, B. C. (1999) J. Am. Soc. Nephrol. 10, 562–568
4. Thomas, W. G. (1999) Regul. Pept. 79, 9–23
5. Hunyady, L., Catt, K. J., Clark, A. J., and Gáborik, Z. (2000) Regul. Pept. 91, 29–44
6. Zhang, J., Ferguson, S. S. G., Barak, I. S., Menard, L., and Caron, M. G. (1996) J. Biol. Chem. 271, 18302–18305
7. Gáborik, Z., Staszak, M., Szondy, L., Balla, B., Poku, S., Catt, K. J., Clark, A. J. L., and Hunyady, L. (2001) Mol. Pharmacol. 59, 239–247
8. Qian, H., Pipolo, L., and Thomas, W. G. (2001) Mol. Endocrinol. 15, 1706–1719
9. Anghorh, P. H., Seachrist, J. L., Dale, L. B., and Ferguson, S. S. G. (2000) Mol. Endocrinol. 14, 2040–2053
10. Kobrut, T. A., Lin, F. S., Perry, S. J., Conner, D. A., and Leffkowitz, R. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 1601–1606
11. McPherson, P. S. (1999) Cell. Signal. 11, 229–238
12. Marsh, M., and McMahon, H. T. (1999) Science 285, 215–220
13. Hinsaw, J. E. (2000) Annu. Rev. Cell Dev. Biol. 16, 483–519
14. Muhlberg, A. B., Warnock, D. E., and Schmid, S. L. (1997) EMBO J. 16, 6676–6683
Role of the Proline-rich Domain of Dynamin-2 and Its Interactions with Src Homology 3 Domains during Endocytosis of the AT$_1$ Angiotensin Receptor
Márta Szaszák, Zsuzsanna Gáborik, Gábor Turu, Peter S. McPherson, Adrian J. L. Clark, Kevin J. Catt and László Hunyady

J. Biol. Chem. 2002, 277:21650-21656.
doi: 10.1074/jbc.M200778200 originally published online March 29, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M200778200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 64 references, 31 of which can be accessed free at http://www.jbc.org/content/277/24/21650.full.html#ref-list-1