THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 39, Issue of September 26, pp. 37545–37552, 2003
Printed in U.S.A.

The Adenosine A_{2A} Receptor Interacts with the Actin-binding Protein α-Actinin*

Received for publication, March 19, 2003, and in revised form, June 27, 2003
Published, JBC Papers in Press, July 1, 2003, DOI 10.1074/jbc.M302809200

Javier Burgueño‡, Derek J. Blake§¶, Matthew A. Benson§‖, Caroline L. Tinsley§, Christopher T. Esapa§, Enric I. Canela‡, Petronila Penela, Josefa Mallol‡, Federico Mayor, Jr.**, Carmen Lluis‡, Rafael Franco‡, and Francisco Ciruela‡ ‡‡**

*From the ‡Department of Biochemistry and Molecular Biology, University of Barcelona, 08028 Barcelona, Spain, the §Department of Pharmacology, University of Oxford, Oxford OX1 3QT, United Kingdom, and the **Centro de Biología Molecular "Severo Ochoa," Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid 28049, Spain*

Recently, evidence has emerged that heptaspanning membrane or G protein-coupled receptors may be linked to intracellular proteins identified as regulators of receptor anchoring and signaling. Using a yeast two-hybrid screen, we identified α-actinin, a major F-actin-cross-linking protein, as a binding partner for the C-terminal domain of the adenosine A_{2A} receptor (A_{2A}R). Colocalization, co-immunoprecipitation, and pull-down experiments showed a close and specific interaction between A_{2A}R and α-actinin in transfected HEK-293 cells and also in rat striatal tissue. A_{2A}R activation by agonist induced the internalization of the receptor by a process that involved rapid β-arrestin translocation from the cytoplasm to the cell surface. In the subsequent receptor traffic from the cell surface, the role of actin organization was shown to be crucial in transiently transfected HEK-293 cells, as actin depolymerization by cytochalasin D prevented its agonist-induced internalization. A_{2A}ΔCTR, a mutant version of A_{2A}R that lacks the C-terminal domain and does not interact with α-actinin, was not able to internalize when activated by agonist. Interestingly, A_{2A}ΔCTR did not show aggregation or clustering after agonist stimulation, a process readily occurring with the wild-type receptor. These findings suggest an α-actinin-dependent association between the actin cytoskeleton and A_{2A}R trafficking.

Adenosine is a molecule that plays a regulatory role in the nervous system, acting presynaptically, postsynaptically, and/or non-synaptically (1). Adenosine mediates its actions through activation of specific G protein-coupled heptaspanning membrane receptors, for which four subtypes have been identified (A_1R,[1] A_{2A}R, A_{2B}R, and A_3R) (2). A_{2A}Rs are coupled mostly to G_s proteins (3), thus being mainly linked to adenylyl cyclase activation. The role of A_{2A}R in the control of neurotransmitter release has been studied in several brain regions as well as in the peripheral nervous system (4). Compared with the other adenosine receptor subtypes, A_{2A}Rs are specially concentrated in the striatopallidal complex (5). Using ultrastructural techniques, Hettinger *et al.* (6) have recently demonstrated that A_{2A}Rs are mostly localized postsynaptically in dendrites and dendritic spines of rat striatal GABAergic (where GABA is γ-aminobutyric acid) neurons. The membrane expression and localization of A_{2A}R are the result of ligand-mediated clustering, internalization, and receptor trafficking (7).

α-Actinin is a major F-actin-cross-linking protein present in both muscle and non-muscle cells. There are four α-actinin genes: two non-skeletal muscle isoforms (α-actinin-1 and -4) and two skeletal muscle isoforms (α-actinin-2 and -3) (8), all of which share a general structure, which can be divided into three functionally distinct domains: the N terminus, which contains two calponin homology domains and which mediates the interaction with actin; a central region composed of four spectrin-like motifs; and the C terminus, which contains EF-hand domains. Recently, the spatial expression of α-actinin-2 in the rat central nervous system was analyzed, revealing that the highest levels of the protein are found in the striatum, cortex, and hippocampus, where it has been shown to interact with the glutamate NMDA receptor (9).

In this study, using a yeast two-hybrid approach and complementary biochemical techniques, we provide evidence for the existence of a physical interaction between A_{2A}R and α-actinin. Furthermore, we also demonstrate that there is a functional association between the actin cytoskeleton and A_{2A}R and more specifically with the receptor internalization process and its membrane agonist-induced aggregation or clustering.

EXPERIMENTAL PROCEDURES

Plasmid Constructs

Two *Eco*RI-*Eco*RI fragments of the C-terminal tail of A_{2A}R were subcloned into the bait vector pHybLexA/Zeo (Invitrogen) and the bacterial expression vector pGEX-4T-1 (Amersham Biosciences). One fragment coding for amino acids 293–412 (LA2A) was amplified using *Taq* DNA polymerase (Sigma) and the following primers: FLA2A (5′-TAA-

* This work was supported in part by Ministerio de Ciencia y Tecnología Grants BIO99-0601-C02-02 and SAF2002-03293 (to R. F.) and SAF2001-3474 (to E. I. C.), Fundació La Marató de TV3 Grant Marató/2001/012710 (to R. F.), and European Community Grant QLG3-CT-2001-01056 (to R. F.). Work performed in the Blake laboratory was funded by the Wellcome Trust. 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.

¶ Wellcome Trust Senior Fellow.
‖ Recipient of a Wellcome Trust prize studentship.
‡‡ Supported by a Ramón y Cajal research contract with the Ministerio de Ciencia y Tecnología. To whom correspondence should be addressed: Dept. de Bioquímica i Biologia Molecular, Universitat de Barcelona, C/Martí i Franquès, 1, 08028 Barcelona, Spain. Tel.: 34-934-021-213; Fax: 34-934-021-219; E-mail: recep@bq.ub.es.

[1] The abbreviations used are: R, receptor; NMDA, *N*-methyl-D-aspartate; GFP, green fluorescent protein; DMEM, Dulbecco's modified Eagle's medium; GST, glutathione *S*-transferase; RIPA, radioimmune precipitation assay; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; MEKK1, MEK kinase-1; MAPK, mitogen-activated protein kinase.

GAATTCCGCGAGTTCCGCCAGACC-3′) and RA2A (5′-CCGGAATTC-CAAGCCAACCAGAAAGATAAAG-3′). For the second fragment coding for amino acids 322–412 (SA2A), the following primers were used: FSA2A (5′-TAAGAATTCCGGGTCTTGGCAGCTCATGGC-3′) and RA2A. The FLAG epitope was introduced between amino acids 6 and 7 of human A_1R using the same PCR mutagenesis approach used for the $A_{2A}R$-FLAG construct (10). A mutant lacking the C-terminal domain of $A_{2A}R$ was generated using $A_{2A}R$-FLAG as the template and the following primers: FA2A (5′-CGGAATTCATGCCCATCATG-3′) and RA2AΔCT (5′-GCTCT-AGACTAGTAGGCGTAGATGAAGGG-3′). The sequences of the cDNAs and their orientation in the vectors were confirmed by DNA sequencing. β-Arrestin-1-GFP, β-arrestin-2-GFP, and β-arrestin-1(S412D) were kindly provided by Drs. J. L. Benovic, M. G. Caron, and R. J. Lefkowitz, respectively.

Yeast Two-hybrid System

Yeast Two-hybrid Screening—A bait strain was created by transforming pHybLex-LA2A into *Saccharomyces cerevisiae* strain L40 following the manufacturer's instructions (Hybrid Hunter, Invitrogen). The bait strain was cotransformed with a mouse brain cDNA library constructed in the Gal4 activating domain vector pPC86 (Invitrogen), and transformants were plated onto minimal yeast medium lacking histidine, tryptophan, uracil, and lysine and containing 300 mg/ml Zeocin (Invitrogen) and 5 mM 3-aminotriazole. Plates were incubated at 30 °C for 5 days, and yeast colonies that grew on histidine-deficient medium were re-streaked onto fresh selective plates and assayed for β-galactosidase activity following the manufacturer's instructions. Prey plasmids were isolated from yeast and electroporated into *Escherichia coli* XL-1Blue electrocompetent cells (Stratagene). The 5′-end of each clone was sequenced using a vector primer. To confirm the interaction in yeast, purified prey plasmids and α-actinin-3 [2] were re-transformed with the pHybLex-LA2A bait and with the empty bait vector pHybLexA/Zeo and tested for growth on selective plates and β-galactosidase activity. Other control yeast strains used were the positive control strain containing pHybLexA/Zeo-dystrobrevin and pYESTrp-syntrophin plasmids and the negative control strain containing the empty bait and prey vectors pHybLexA/Zeo and pYESTrp2 (Invitrogen).

Liquid β-Galactosidase Assay—1.5 ml of each culture (grown for 48 h at 30 °C) was spun, and the pellet was resuspended in 200 μl of Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, and 1 mM $MgSO_4$, pH 7.0). A small amount of glass beads (425–600 μm; Sigma) was added, and the mixture was sonicated for 5–10 min. After cell lysis, the samples were spun to pellet the cell debris. 100 μl of supernatant was transferred to a new microcentrifuge tube, and 700 μl of Z buffer containing β-mercaptoethanol (27 μl/10 ml) was added. 150 μl of 2.5 mg/ml *o*-nitrophenyl-β-D-galactopyranoside (Sigma) was added to the sample, and the mixture was incubated at 37 °C for 3 h. The absorbance was read at 420 nm and refers to the amount of protein present in each sample.

Cell Culture and Transfection

HEK-293 cells were grown in DMEM (Sigma) supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, 100 units/ml penicillin/streptomycin, and 10% (v/v) fetal bovine serum at 37 °C in an atmosphere of 5% CO_2. HEK-293 cells growing in 25-cm^2 dishes or 20-mm coverslips were transiently transfected with 10 μg of DNA coding for the proteins specified in each case by calcium phosphate precipitation (11). The cells were harvested at either 24 or 48 h after transfection.

For primary cultures, striatal tissue from Sprague-Dawley rat embryos was treated with trypsin (0.5 g/liter) and EDTA (2 g/liter) solution (Sigma) for 10 min at 37 °C. Trypsinization was stopped by addition of complete medium (DMEM containing Glutamax-I, 10% fetal calf serum, and 50 μg/ml gentamycin), and tissue was gently triturated (10 passages) with a flame-sterilized glass Pasteur pipette. Cells were plated at $4–5 \times 10^4$ cells/cm^2 in DMEM containing Glutamax-I, 10% heat-inactivated fetal bovine serum, and 50 μg/ml gentamycin at 5% CO_2 in poly-D-lysine-coated glass coverslips, and the medium was replaced after 1 h with DMEM containing 10% heat-inactivated FCS and 50 μg/ml gentamycin. The next day, the cells were washed with DMEM, and the medium was replaced with serum-free B27-supplemented neurobasal medium (Invitrogen) containing 50 μg/ml gentamycin. The cells were kept at 37 °C in an atmosphere of 5% CO_2 for 2–3 weeks without frequent medium change.

[2] D. J. Blake, manuscript in preparation.

Gel Electrophoresis and Immunoblotting

SDS-PAGE was performed using 7.5 or 10% polyacrylamide gels. Proteins were immunoblotted onto polyvinylidene difluoride membranes (Immobilon-P, Millipore Corp.) using a semidry transfer system and developed with an enhanced chemiluminescence detection kit (Pierce) as described previously (12).

Expression of GST Fusion Proteins and Pull-down Assay

GST, GST-SA2A, and GST-LA2A recombinant fusion proteins were expressed in *E. coli* strain BL21 (Promega) with 0.1 mM isopropyl-β-D-thiogalactopyranoside (Sigma) for 3 h at 37 °C and purified on glutathione-Sepharose (Amersham Biosciences) as described previously (12). 5 μg of each protein was coupled to 100 μl of a 50% (v/v) suspension of glutathione-agarose beads (Sigma) in phosphate-buffered saline for 1 h at 4 °C. Human embryonic kidney cells (HEK-293) were solubilized in ice-cold RIPA buffer (50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 0.5% sodium deoxycholate, 0.2% SDS, 100 mM NaCl, and 1 mM EDTA) for 30 min at 4 °C. The solubilized material was centrifuged at 14,000 rpm for 20 min, and the supernatant was precleared with 100 μl of the 50% (v/v) suspension of glutathione-agarose beads for 1 h with constant rotation at 4 °C. After the preclearing, supernatants were transferred to a clean tube containing GST, GST-SA2A, or GST-LA2A coupled to glutathione-agarose and incubated overnight with constant rotation at 4 °C. Subsequently, the beads were washed twice with ice-cold lysis buffer (phosphate-buffered saline, pH 7.4, containing 1% (v/v) Nonidet P-40); twice with 0.1% (v/v) of ice-cold RIPA buffer; and once with 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 1 mM EDTA. Then, 50 μl of SDS-PAGE sample buffer was added to each sample. Bound proteins were dissociated by heating at 37 °C for 1 h and resolved by SDS-PAGE on 7.5% gels as described above.

Antibodies

An antiserum against the GST fusion protein containing amino acids 322–412 (SA2A) of $A_{2A}R$ (designated as anti-CTA2A) was used in this study. Immunization of rabbits and affinity purification of the antisera were performed as described previously (12, 13). Other primary antibodies used were as follows: anti-calnexin monoclonal antibody (Transduction Laboratories), anti-FLAG monoclonal antibody (clone M2; Sigma), anti-α-actinin polyclonal antibody (Santa Cruz Biotechnology), anti-α-actinin monoclonal antibody (Sigma), and anti-β-arrestin polyclonal antibody Ab186 (1:1000 dilution) (14). The secondary antibodies used were as follows: horseradish peroxidase-conjugated goat anti-rabbit IgG (1:60,000 dilution; Pierce), horseradish peroxidase-conjugated rabbit anti-mouse IgG (1:2000 dilution; Dako Corp.), Alexa 488-conjugated goat anti-mouse IgG (1:1000 dilution) and Texas Red-conjugated goat anti-rabbit IgG (1:2000 dilution; both from Molecular Probes, Inc.), and Cy3-conjugated donkey anti-mouse IgG (1:250 dilution; Jackson ImmunoResearch Laboratories, Inc.).

Immunoprecipitation

Transfected HEK-293 cells were solubilized with ice-cold RIPA buffer, and lysates were incubated with anti-FLAG monoclonal antibody (2 μg/ml) or anti-CTA2A polyclonal antibody (2 μg/ml) for 2 h. Then, 40 μl of a suspension of protein G or protein A cross-linked to agarose beads (Sigma) was added, and the mixture was incubated overnight with constant rotation at 4 °C. The beads were washed twice with ice-cold RIPA buffer; twice with 0.1% (v/v) of ice-cold RIPA buffer; and once with 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 1 mM EDTA. Subsequently, 50 μl of SDS-PAGE sample buffer was added to each sample. Immune complexes were dissociated by heating to 37 °C for 1 h and resolved by SDS-PAGE on 7.5 or 10% gels as described above.

Biotinylation of Cell-surface Proteins

HEK-293 cells transiently transfected with $A_{2A}R$-FLAG or A_{2A}ΔCTR-FLAG were washed three times with borate buffer (10 mM H_3BO_3, pH 8.8, and 150 mM NaCl) and then incubated with 50 μg/ml sulfosuccinimidyl 6-(biotinamido)hexanoate (Pierce) in borate buffer for 5 min at room temperature. Cells were washed three times with borate buffer and again incubated with 50 μg/ml sulfosuccinimidyl 6-(biotinamido)hexanoate in borate buffer for 10 min at room temperature, and 100 mM NH_4Cl was added for 5 min to quench the remaining biotin. Cells were washed with Tris-buffered saline, disrupted with three 10-s strokes in a Polytron, and centrifuged at 14,000 rpm for 30 min. The pellet was solubilized in ice-cold lysis buffer for 30 min and centrifuged at 14,000 rpm for 20 min. The supernatant was incubated with 80 μl of streptavidin-agarose beads (Sigma) for 1 h with constant rotation at 4 °C. Beads were washed twice with ice-cold lysis buffer, twice with

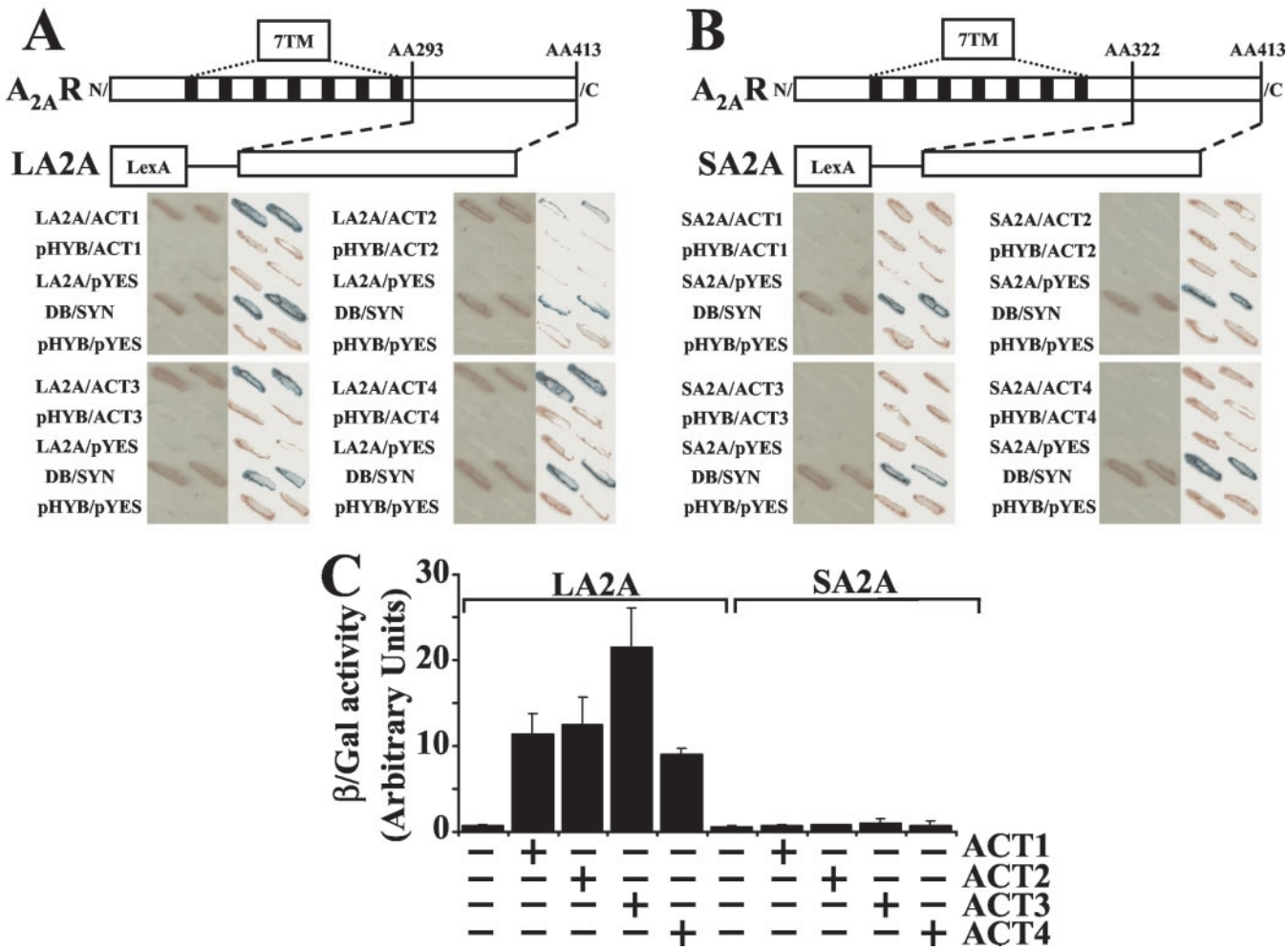

FIG. 1. **α-Actinin interacts with $A_{2A}R$ in the yeast two-hybrid system.** *A*, schematic representation of the pHybLex-LA2A (*LA2A*) fusion protein containing amino acids (*AA*) 293–412 of the C-terminal tail of $A_{2A}R$ (*upper panel*). Shown are the results from β-galactosidase filter assays and growth assays on yeast cotransformed with the bait and prey plasmids indicated (*lower panels*). *B*, schematic representation of the pHybLex-SA2A (*SA2A*) fusion protein containing amino acids 322–412 of the C-terminal tail of $A_{2A}R$ (*upper panel*). Shown are the results from β-galactosidase filter assays and growth assays on yeast cotransformed with the bait and prey plasmids indicated (*lower panels*). In *A* and *B*, yeast cells containing interacting clones are indicated by their growth on histidine-deficient medium and by the *blue* color corresponding to β-galactosidase activity. *C*, quantitation of the interaction of each α-actinin isoform with $A_{2A}R$ fusion proteins determined using a liquid β-galactosidase (*β/Gal*) assay as described under "Experimental Procedures." Data are means ± S.E. of three replicates. *ACT1*, *ACT2*, *ACT3*, and *ACT4*, α-actinin-1, -2, -3, and -4, respectively; *DB*, α-dystrobrevin; *SYN*, syntrophin; *pHYB*, pHybLex; *pYES*, pYESTrp2; *7TM*, seven-transmembrane domain.

0.1% (v/v) of ice-cold lysis buffer, and once with ice-cold phosphate-buffered saline. The complexes were dissociated by adding 50 μl of SDS-PAGE sample buffer, heated at 37 °C for 1 h, and then resolved by SDS-PAGE on 10% gels. The gels were run and immunoblotted as described above.

Immunocytochemistry

Transiently transfected HEK-293 cells or rat striatal primary cultures were fixed in 4% paraformaldehyde for 15 min and washed with phosphate-buffered saline containing 20 mM glycine (buffer A) to quench the remaining free aldehyde groups. Cells were permeabilized with buffer A containing 0.2% Triton X-100 for 5 min. Blocking was done using buffer A containing 1% bovine serum albumin (buffer B). Cells were labeled for 1 h at room temperature with the indicated primary antibody, washed for 30 min with buffer B, and stained with the corresponding secondary antibody for 1 h. Coverslips were rinsed for 30 min with buffer B and mounted with Immuno Fluore mounting medium (ICN). An Olympus FV 300 scanning laser confocal microscope was used for observations.

RESULTS

Yeast Two-hybrid Screening—To identify intracellular proteins interacting with $A_{2A}R$, the last 120 amino acids of $A_{2A}R$ were fused in-frame with LexA in the pHybLexA/Zeo vector (LA2A) (Fig. 1*A*) and used to screen a mouse brain cDNA library with the yeast two-hybrid system. Of the 24 clones (from the 2×10^6 total transformants screened that were found to grow on nutritionally deficient plates and activated the β-galactosidase assay), seven were identified as different isoforms of the actin-binding protein α-actinin: three independent clones for α-actinin-1, another three for α-actinin-4, and one clone for α-actinin-2. To discard a false-positive interaction, all four different α-actinin isoforms were transformed again with LA2A and plated along with different negative and positive controls. As shown in Fig. 1*A*, only those transformants containing the C-terminal tail of $A_{2A}R$ and any of the α-actinins could grow on deficient medium and turned blue in the β-galactosidase assay, as did the positive control dystrobrevin/syntrophin (*DB*/*SYN*) (15). To determine the region of the C-terminal domain of $A_{2A}R$ that interacts with α-actinin, another LexA fusion protein of the last 91 amino acids of the receptor was constructed (SA2A) (Fig. 1*B*) and tested for its ability to bind α-actinin. This shorter fusion protein could not interact with any of the α-actinin isoforms (Fig. 1, *B* and *C*), thus mapping the interacting domain within amino acids 293–321 of $A_{2A}R$. The interaction of both the LA2A and SA2A constructs and α-actinins was quantitated using a liquid β-galactosidase

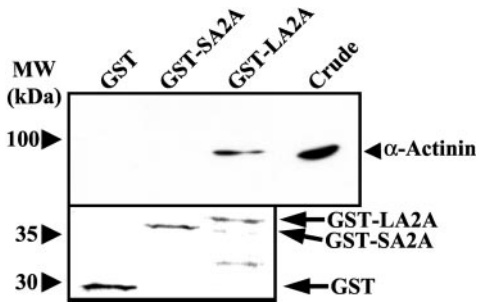

FIG. 2. **GST-LA2A and GST-SA2A pull-down experiments.** Endogenously expressed α-actinin in HEK-293 cell extracts (*Crude*) was pulled down with GST-LA2A, but not with GST-SA2A or GST alone. α-Actinin was detected using a polyclonal antibody against α-actinin (1:500 dilution). The primary bound antibody was detected using a horseradish peroxidase-conjugated goat anti-rabbit antibody (1:60,000 dilution). The *lower panel* shows the relative amounts of GST, GST-SA2A, and GST-LA2A fusion proteins used in the pull-down experiment stained with Coomassie Blue.

assay (Fig. 1*C*), showing the clearly different binding abilities of both $A_{2A}R$ constructs and confirming the region between amino acids 293 and 321 as the α-actinin-interacting domain.

α-Actinin Binds to the C-terminal Domain of $A_{2A}R$—To verify the interaction between α-actinin and the C-terminal tail of $A_{2A}R$, we tested the ability of naturally expressed full-length α-actinin to associate with GST fusion proteins containing the LA2A and SA2A C-terminal regions of $A_{2A}R$ (GST-LA2A and GST-SA2A). As shown in Fig. 2, an immunoreactive band of 100 kDa corresponding to α-actinin endogenously expressed by HEK-293 cells could be detected in the crude extracts from these cells. This band was observed in pull-down assays when cell lysates were incubated with GST-LA2A fusion protein, but was not detected either with GST-SA2A fusion protein or with GST alone (Fig. 2). This result shows that naturally expressed α-actinin binds specifically to a region in $A_{2A}R$ between amino acids 293 and 321.

Interaction of $A_{2A}R$ and α-Actinin in Transfected HEK-293 Cells—The association of $A_{2A}R$ and α-actinin was subsequently studied in transfected HEK-293 cells by double immunolabeling and co-immunoprecipitation experiments. By confocal microscopy analysis of HEK-293 cells transiently transfected with the cDNA coding for $A_{2A}R$-FLAG, a marked overlap in the distribution of the two proteins was found (Fig. 3*A*). Interestingly, when the double immunolabeling experiment was performed in HEK-293 cells transiently transfected with the cDNA coding for A_1R-FLAG (another adenosine receptor subtype with a shorter C-terminal tail) or with the cDNA coding for a mutant of $A_{2A}R$ lacking the whole C-terminal domain (A_{2A}ΔCTR-FLAG), no colocalization with α-actinin was observed (Fig. 3*A*), suggesting a specificity for the colocalization between $A_{2A}R$-FLAG and α-actinin.

When cell extracts of HEK-293 cells transiently transfected with $A_{2A}R$-FLAG were immunoprecipitated with an antibody against the FLAG epitope and these immunoprecipitates were analyzed by Western blotting using an antibody against α-actinin, a band of 100 kDa corresponding to α-actinin was observed (Fig. 3*B*, *lane 4*). Interestingly, this band did not appear in immunoprecipitates from cells transfected with the cDNAs coding for LacZ (Fig. 3*B*, *lane 1*), A_1R-FLAG (*lane 2*), and A_{2A}ΔCTR-FLAG (*lane 3*). These results demonstrate that α-actinin can interact with full-length $A_{2A}R$; that the interaction is mediated by the C-terminal tail of $A_{2A}R$; and that the $A_{2A}R$/α-actinin interaction is receptor subtype-specific, as A_1R did not interact with α-actinin.

Interaction of $A_{2A}Rs$ with α-Actinin in Rat Striatal Homogenates and Primary Rat Striatal Neurons—To assess the physiological relevance of the $A_{2A}R$/α-actinin interaction, we performed co-immunoprecipitation experiments with rat striatal homogenates and double immunolabeling with primary cultures of rat striatal neurons. Using soluble extracts from rat striatum that had been shown by Western blotting to contain both α-actinin and $A_{2A}R$ (Fig. 4*A*, *lane 1*), anti-CTA2A antibody could immunoprecipitate a band of ~100 kDa that was detected with α-actinin antibody (*lane 2*). This band did not appear when protein A-agarose was used for immunoprecipitation (Fig. 4*A*, *lane 3*), showing that there was no unspecific interaction between α-actinin and the resin.

The distribution of α-actinin and $A_{2A}R$ in primary rat striatal neurons was also tested. By confocal microscopy analysis, a similar punctate distribution and a partial colocalization for both proteins were found (Fig. 4*B*). Colocalization was not

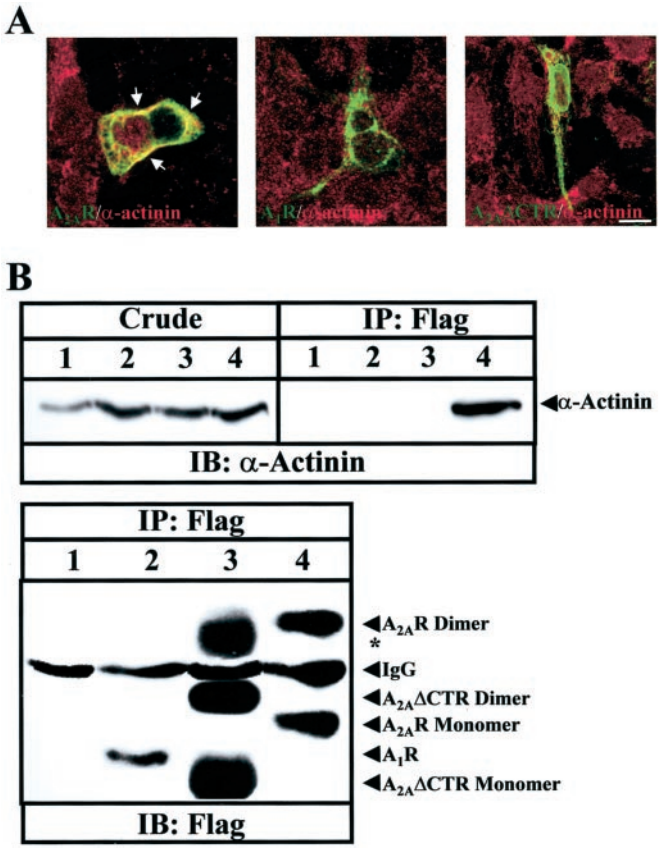

FIG. 3. **Colocalization and co-immunoprecipitation of $A_{2A}R$ and α-actinin in HEK-293 cells.** *A*, HEK-293 cells transiently transfected with $A_{2A}R$-FLAG, A_1R-FLAG, or A_{2A}ΔCTR-FLAG and fixed and permeabilized as described under "Experimental Procedures" were labeled with anti-FLAG monoclonal antibody (1:200 dilution) and anti-α-actinin polyclonal antibody (1:100 dilution), followed by Texas Red-conjugated goat anti-rabbit (1:2000 dilution) and Alexa 488-conjugated goat anti-mouse (1:1000 dilution) antibodies. Cells were analyzed by double immunofluorescence with a confocal microscope. Superimposition of images revealed colocalization of α-actinin with $A_{2A}R$-FLAG (*yellow*, *arrows*), but not with A_1R-FLAG or A_{2A}ΔCTR-FLAG. *Scale bar* = 10 μm. *B*, anti-FLAG monoclonal antibody (2 μg/ml) was used to immunoprecipitate (*IP*) $A_{2A}R$-FLAG, A_1R-FLAG, and A_{2A}ΔCTR-FLAG receptors transiently transfected in HEK-293 cells. The crude extracts and immunoprecipitates were analyzed by SDS-PAGE and immunoblotted (*IB*) using a polyclonal antibody against α-actinin (1:500 dilution) and a monoclonal antibody against the FLAG epitope (1:500 dilution). α-Actinin was detected in crude extracts from HEK-293 cells transfected with LacZ (*lane 1*), A_1R-FLAG (*lane 2*), A_{2A}ΔCTR-FLAG (*lane 3*), or $A_{2A}R$-FLAG (*lane 4*), but it was detected only in those immunoprecipitates of $A_{2A}R$-FLAG-expressing cells. The *lower panel* shows the ability of anti-FLAG antibody to immunoprecipitate A_1R-FLAG (*lane 2*), A_{2A}ΔCTR-FLAG (*lane 3*), and $A_{2A}R$-FLAG (*lane 4*) receptors from their respective extracts. The *asterisk* indicates a higher molecular mass species of the A_{2A}ΔCTR-FLAG receptor.

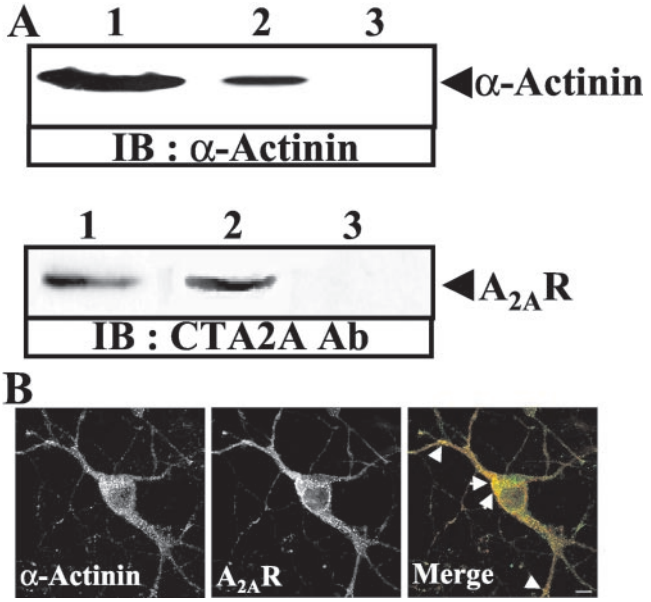

FIG. 4. ***In vivo* interaction of $A_{2A}R$ and α-actinin in rat striatum.** *A*, solubilized extracts from rat striatum were subjected to immunoprecipitation analysis using anti-CTA2A antibody (2 μg/ml). Extracts (*lane 1*) and immunoprecipitates (*lane 2*) were analyzed by SDS-PAGE and immunoblotted (*IB*) using anti-α-actinin polyclonal antibody (1:500 dilution) (*upper panel*) and the polyclonal antibody against $A_{2A}R$, anti-CTA2A antibody (*Ab*; 1:2000 dilution) (*lower panel*). Both $A_{2A}R$ and α-actinin could be detected in the crude extract (*lane 1*) and in the immunoprecipitate (*lane 2*) with anti-CTA2A antibody, but they could not be detected with protein-A agarose alone (*lane 3*). *B*, primary cultures of rat striatal neurons (days *in vitro* 14–21) were cultured and processed for immunolabeling as described under "Experimental Procedures" using affinity-purified anti-CTA2A polyclonal antibody (1:50 dilution) and anti-α-actinin monoclonal antibody (1:100 dilution). Cells were analyzed by double immunofluorescence with a confocal microscope. Superimposition of images revealed colocalization (*yellow*) both in the cell body (*arrows*) and in dendrites (*arrowheads*). *Scale bar* = 10 μm.

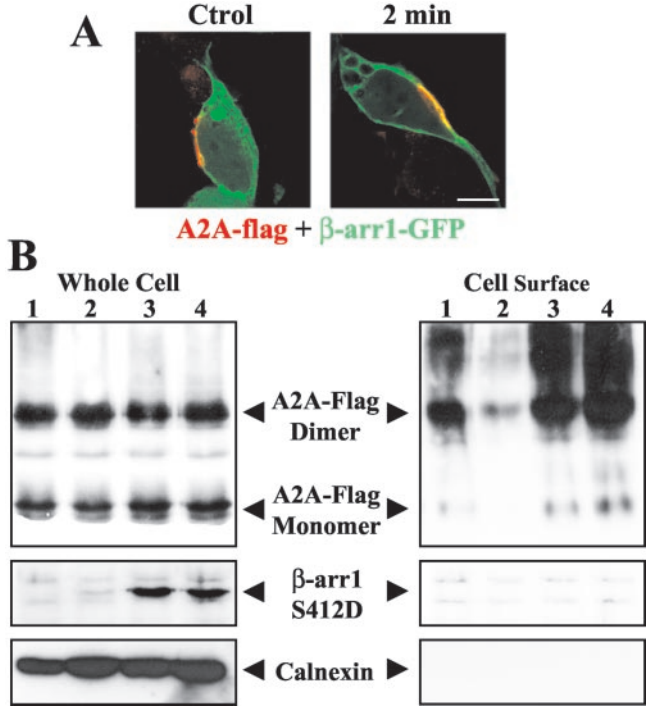

FIG. 5. **Ligand-induced $A_{2A}R$ internalization.** *A*, HEK-293 cells transiently transfected with $A_{2A}R$-FLAG (*A2A-flag*) plus β-arrestin-1-GFP (*β-arr1-GFP*) were treated with vehicle (control (*Ctrol*)) or 200 nM CGS-21680 for 2 min (*2 min*) at 37 °C. Then, cells were fixed as described under "Experimental Procedures" and labeled with anti-FLAG monoclonal antibody (1:200 dilution), followed by Cy3-conjugated donkey anti-mouse antibody (1:1000 dilution). Cells were analyzed by double immunofluorescence with a confocal microscope. *Scale bar* = 10 μm. *B*, HEK-293 cells transiently transfected with $A_{2A}R$-FLAG (*lanes 1* and *2*) or $A_{2A}R$-FLAG plus β-arrestin-1(S412D) (*βarr1 S412D*) (*lanes 3* and *4*) were treated with vehicle (*lanes 1* and *3*) or with 200 nM CGS-21680 (*lanes 2* and *4*) for 2 h at 37 °C. Cell-surface labeling was performed as described under "Experimental Procedures." Crude extracts and biotinylated proteins were subsequently analyzed by SDS-PAGE and immunoblotted using anti-CTA2A polyclonal antibody (1:2000 dilution), anti-β-arrestin polyclonal antibody Ab186 (1:1000 dilution), and anti-calnexin monoclonal antibody (1:250 dilution).

general, but it occurred both in the cell body, where the degree of colocalization of $A_{2A}R$ with α-actinin was very high (Fig. 4*B*, *arrows*), and at specific aggregates in dendrites (*arrowheads*). These observations are consistent with the idea that α-actinin and $A_{2A}R$ associate in striatal neurons.

Correlation between α-Actinin Binding and $A_{2A}R$ Ligand-induced Trafficking—Because of the role of α-actinin in organizing and cross-linking actin filaments, we analyzed the possibility that the interaction between α-actinin and $A_{2A}R$ reported in this study could be related to the trafficking of the receptor, both its clustering and internalization processes occurring upon agonist stimulation. Although the agonist CGS-21680 (2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine) produces a marked internalization of $A_{2A}R$ (7), the molecules involved in this process are not well known. For example, the role of β-arrestin proteins in the internalization process of $A_{2A}R$ has not yet been described. Confocal scanning microscopy was used to visualize in cotransfected cells the possible translocation of β-arrestin-1-GFP or β-arrestin-2-GFP from the cytoplasm to the cell surface. Fig. 5*A* shows that, in the absence of agonist, β-arrestin-1-GFP was homogeneously localized in the cytosol of cells also expressing $A_{2A}R$-FLAG. Upon stimulation with 200 nM CGS-21680, a rapid (2 min) translocation of β-arrestin-1-GFP to the cell surface was observed. Similar results were obtained when $A_{2A}R$ was cotransfected with β-arrestin-2-GFP (data not shown).

To further examine whether $A_{2A}R$ internalization is arrestin-dependent, the receptor was cotransfected with β-arrestin-1(S412D), a mutant with dominant-negative properties with respect to receptor internalization (16). To analyze the amount of receptor in the plasma membrane, we isolated cell-surface proteins by protein biotinylation using a membrane-impermeant biotin ester. Proteins isolated by streptavidin-agarose affinity precipitation were analyzed by SDS-PAGE and immunoblotted with antibodies against the C-terminal tail of $A_{2A}R$ (anti-CTA2A) and the endoplasmic reticulum-associated protein calnexin. The absence of calnexin in the streptavidin isolates indicated that the biotin ester had not penetrated into the cell and that it labeled only proteins in the plasma membrane (Fig. 5*B*, *lower panels*). As shown in Fig. 5*B*, the overexpressed arrestin mutant inhibited agonist-induced $A_{2A}R$ internalization, indicating that $A_{2A}R$ is able to recruit β-arrestin proteins to the plasma membrane and that its agonist-dependent internalization process is, as in the case of other G protein-coupled receptors of the same family, β-arrestin-dependent (17, 18). The effect of α-actinin in the internalization process subsequent to β-arrestin recruitment was determined by analyzing the need of an intact actin cytoskeleton for receptor internalization. HEK-293 cells transiently transfected with $A_{2A}R$-FLAG were pretreated with vehicle (Fig. 6, *A* and *B*) or with 2 μM cytochalasin D for 2 h (*C* and *D*) as described previously (19). After treatment, cells were incubated with vehicle (Fig. 6, *A* and *C*, *lane 1*) or with 200 nM CGS-21680 (*A* and *C*, *lane 2*) for 2 h at 37 °C. After internalization, receptor recycling was assessed by washing the cells after agonist treatment and further

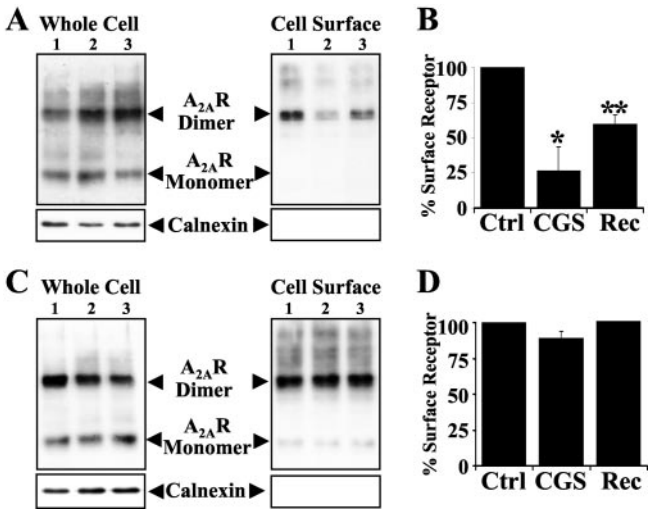

FIG. 6. **Effect of cytochalasin D on agonist-induced internalization of $A_{2A}R$.** HEK-293 cells transiently transfected with $A_{2A}R$-FLAG were pretreated in the absence (*A*) or presence (*C*) of 2 μM cytochalasin D for 2 h and then treated with vehicle (*lane 1*) or with 200 nM CGS-21680 (*lanes 2* and *3*) for 2 h at 37 °C. To assess receptor recycling (*Rec*) to the cell surface, cells were washed and incubated with fresh medium for an extra hour at 37 °C (*lane 3*). Cell-surface labeling was performed as described under "Experimental Procedures." Crude extracts and biotinylated proteins were subsequently analyzed by SDS-PAGE and immunoblotted using anti-CTA2A polyclonal antibody (1:2000 dilution) and anti-calnexin monoclonal antibody (1:250 dilution). *B* and *D* are relative densitometric scans of the immunoblots in *A* and *C*, respectively. Cell-surface receptor values were normalized using the total amount of receptor in the crude extract for each sample. The results are presented as means ± S.E. of three independent experiments. *, $p < 0.01$ *versus* control (*Ctrl*) cells; **, $p < 0.05$ *versus* CGS-21680 (*CGS*)-treated cells.

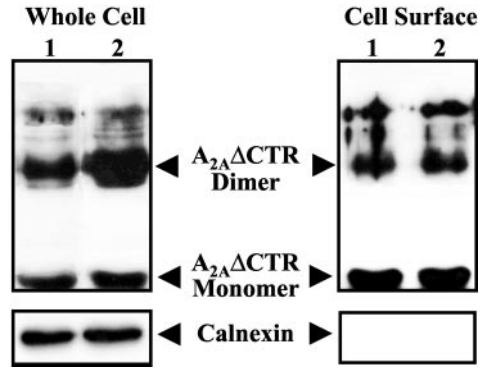

FIG. 7. **The C-terminal tail of $A_{2A}R$ is directly involved in its agonist-induced internalization.** HEK-293 cells transiently transfected with A_{2A}ΔCTR-FLAG were treated with vehicle (*lane 1*) or with 200 nM CGS-21680 (*lane 2*) for 2 h at 37 °C. After this incubation period, cells were processed for cell-surface biotinylation as described under "Experimental Procedures." Crude extracts and cell-surface biotinylated proteins were subsequently analyzed by SDS-PAGE and immunoblotted using anti-FLAG monoclonal antibody (1:500 dilution) and anti-calnexin monoclonal antibody (1:250 dilution).

incubation for 1 h at 37 °C with fresh medium (Fig. 6, *A* and *C*, *lane 3*). The results show that the amount of receptor present in the cell surface decreased substantially in cells treated with CGS-21680 (Fig. 6*A*, *lane 2*) compared with control cells (*lane 1*). When the agonist was washed out, the cell-surface expression of the receptor was partially recovered after 1 h (Fig. 6*A*, *lane 3*). In contrast, pretreatment of cells with cytochalasin D avoided agonist-induced internalization of $A_{2A}R$ (Fig. 6*C*, *lane 2*). Quantitation of the cell surface-associated receptor by densitometric scanning of the immunoreactive bands on the films indicated that agonist treatment or challenge induced an internalization of 74% of the total cell-surface receptor (Fig. 6*B*). Under the same conditions, when cells were pretreated with the actin-disrupting agent cytochalasin D, the agonist challenge did not induce internalization of the receptor (Fig. 6*D*). These results indicate that a completely organized actin cytoskeleton is necessary for the agonist-dependent internalization of $A_{2A}R$. Moreover, the internalization ability of A_{2A}ΔCTR, the C-terminal deletion mutant of $A_{2A}R$ that showed neither colocalization nor co-immunoprecipitation with α-actinin (Fig. 3), was tested. After agonist treatment of transiently transfected cells with A_{2A}ΔCTR-FLAG, the membrane proteins were isolated (see above) and analyzed by immunoblotting using anti-FLAG antibody to detect A_{2A}ΔCTR-FLAG (Fig. 7). The results show that the amount of receptor present at the plasma membrane remained invariable after agonist treatment (Fig. 7, *lane 2*) compared with the untreated cells (*lane 1*). Again, the absence of calnexin in the streptavidin isolates indicated that the biotin could not penetrate the cell (Fig. 7, *lower panels*).

Proper attachment of dopamine D_2 receptors to the actin cytoskeleton through the actin-binding protein filamin A has been found to affect cell-surface clustering of this receptor (20). To analyze the plasma membrane expression pattern of $A_{2A}R$ as a function of its ability to bind α-actinin, immunofluorescence experiments with $A_{2A}R$-FLAG- and A_{2A}ΔCTR-FLAG-transfected HEK-293 cells were performed. $A_{2A}R$-FLAG was shown to be uniformly expressed at the cell surface of HEK-293 cells (Fig. 8, *Control*). After 15 min of agonist treatment, a marked aggregation or clustering of $A_{2A}R$-FLAG at the cell-surface level was observed (Fig. 8, *15 min*). Moreover, when cells were incubated for 2 h with the agonist, both cell-surface clustering and a reduction in the total amount of plasma membrane receptor were observed (Fig. 8, *2 h*). These results agree with the biotinylation ones (Fig. 6). Similarly, A_{2A}ΔCTR-FLAG showed an even distribution at the plasma membrane level when expressed in HEK-293 cells (Fig. 8, *Control*). Interestingly, when treated with the agonist, the cell-surface distribution and amount of A_{2A}ΔCTR-FLAG were not altered compared with the control cells (Fig. 8). This agrees with the previous biotinylation results (Fig. 7). Overall, these results indicate that the C-terminal domain of $A_{2A}R$ is essential for agonist-induced clustering and internalization and suggest that a proper attachment to the α-actinin/actin cytoskeleton may be crucial for $A_{2A}R$ trafficking.

DISCUSSION

A yeast two-hybrid approach was used to identify a novel interaction between the heptaspanning membrane adenosine A_{2A} receptor and the actin-cross-linking protein α-actinin. The interaction was verified by pull-down experiments using GST and $A_{2A}R$-GST fusion constructs and by colocalization and co-immunoprecipitation experiments in transfected HEK-293 cells. Moreover, colocalization of both proteins in rat striatal primary cultures and the ability of anti-$A_{2A}R$ antibodies to immunoprecipitate α-actinin from rat striatal homogenates suggest that the interaction is physiologically relevant.

α-Actinin is a component of the actin cytoskeleton that plays a central role by directly cross-linking the actin molecules, so the presence of a complex involving $A_{2A}R$ and α-actinin suggests that α-actinin may mediate receptor association with the actin cytoskeleton. Recent studies have identified filamin A, another actin-cross-linking protein similar to α-actinin, as an intracellular binding partner for other heptaspanning membrane receptors, *viz.* the dopamine D_2 and D_3 receptors (20, 21), the calcium-sensing receptor (22), and metabotropic glutamate receptors (23). A role for these interactions in the proper membrane targeting and synaptic localization of all these receptors has been proposed. Filamin A plays a particularly important

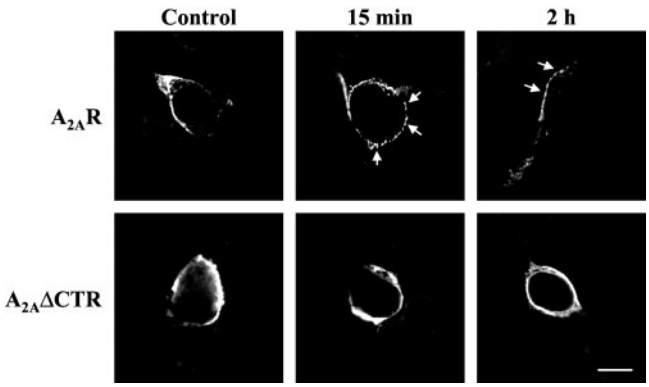

FIG. 8. **$A_{2A}R$ agonist-induced clustering.** HEK-293 cells transiently transfected with $A_{2A}R$-FLAG or $A_{2A}\Delta CTR$-FLAG were treated with vehicle (*Control*) or with CGS-21680 for 15 min (*15 min*) or 2 h (*2 h*). Cells were fixed and labeled with anti-FLAG monoclonal antibody (1:200 dilution), followed by Alexa 488-conjugated goat anti-mouse antibody (1:1000 dilution). Cell-surface receptor immunofluorescence was analyzed with a confocal microscope. *Arrows* indicate receptor aggregates. *Scale bar* = 10 μm.

role in dopamine D_2 receptor plasma membrane expression (21) and cell-surface clustering (20). Similarly, one important result achieved in this work is that the attachment of $A_{2A}R$ to the actin cytoskeleton through α-actinin was revealed as a prerequisite for its agonist-induced plasma membrane clustering.

Although we present here the first evidence for binding of α-actinin to a heptaspanning membrane receptor, it has already been shown to interact with other types of cell receptors such as the ATP-gated ion channel $P2X_7$ (24) and glutamate NMDA receptors, which are clustered and localized at postsynaptic sites by interactions with postsynaptic density protein-95 (25) and α-actinin-2 (26). An NMDA receptor and a α-actinin-2 has been reported in rat cortex and striatum (9), specially in the latter, where both α-actinin-2 mRNA and protein were shown to accumulate. Compared with other adenosine receptors, $A_{2A}R$ is also specially concentrated in the striatum, where we have found a close association with α-actinin by colocalization and co-immunoprecipitation studies. It should be noted that, in rat striatal neurons, NMDA currents are inhibited by the $A_{2A}R$ agonist CGS-21680. This inhibition was shown to be dependent on an intact α-actinin/actin cytoskeleton, as treatment with the actin-depolymerizing agent cytochalasin B prevents $A_{2A}R$-induced NMDA current inhibition (27). Hence, the actin cytoskeleton (and α-actinin particularly) most likely has a role in the functional regulation of NMDA action by $A_{2A}R$ in rat striatum.

There is a growing body of evidence suggesting that actin plays a role in endocytosis (28, 29). Using drugs that inhibit proper actin polymerization, Lunn *et al.* (30) abrogated the internalization of two heptaspanning membrane receptors, *viz.* bombesin and endothelin receptors and the tyrosine kinase receptor for epidermal growth factor. As receptor-mediated endocytosis is a critical phenomenon in receptor down-regulation, ligand degradation, and signal termination, we were interested in the potential role of actin in agonist-dependent $A_{2A}R$ internalization. In this work, a β-arrestin-dependent mechanism for $A_{2A}R$ agonist-induced internalization was demonstrated. $A_{2A}R$ was able to rapidly recruit β-arrestins to the cell surface upon agonist challenge, and a negative mutant of β-arrestin was shown to inhibit agonist-induced $A_{2A}R$ internalization. In this study, we examined the effect of cytochalasin D, an agent that caps the growing end of actin filaments and thus disrupts actively turning over actin stress fibers (31), on the endocytosis of $A_{2A}R$ that occurs after β-arrestin recruitment. Our results show that, whereas $A_{2A}R$ can normally internalize in HEK-293 cells transiently transfected with $A_{2A}R$-FLAG, the exposure of these cells to cytochalasin D inhibits the internalization of the receptor, confirming a functional relationship between $A_{2A}R$ and the underneath actin structure and suggesting that this internalization process depends on actively turning over actin stress fibers rather than on cortical actin, which remains less affected by the action of cytochalasin D (30, 31). Moreover, a mutant of $A_{2A}R$ lacking the C-terminal domain, which could not interact with α-actinin in transfected HEK-293 cells, did not internalize upon agonist exposure. These results show that the C-terminal tail of $A_{2A}R$ is an essential domain for its internalization; and as this domain mediates the interaction with α-actinin, they suggest that a proper attachment of the receptor to the α-actinin/actin cytoskeleton is a prerequisite for its agonist-induced β-arrestin-mediated internalization.

As recently proposed for other G protein-coupled receptors, once internalized, the interaction between receptors and β-arrestins also triggers the ERK cascade, where β-arrestins can behave as agonist-regulated molecular adapters and scaffolds (32), thus allowing activated ERKs to target specific subcellular domains (33). α-Actinin also interacts with proteins involved in signal transduction such as the MEK activator MEKK1 (34) and ERK (35). Recently, we reported that $A_{2A}R$ can signal through the extracellular signal-regulated MAPK cascade (10). Taking all this evidence together, it seems that, apart from β-arrestins, α-actinin has a dual role as an actin cytoskeleton component and as a scaffolding protein, anchoring receptors to their target signaling molecules and so ensuring a rapid and efficient signal transduction. A similar hypothesis has been suggested for another actin-cross-linking protein, filamin A, as this protein interacts with MEK1/2 and p38 kinases (36) and the Ras-related GTPases Rac, RhoA, Cdc42, and RalA (37). So, consistent with a double function as a scaffolding and adapter protein, the interaction of filamin A increases the coupling efficiency of the dopamine D_2 receptor and adenylate cyclase (20, 21). Moreover, α-actinin-2 competes with calmodulin for binding to the C-terminal domain of the NMDA NR1 subunit, which is involved in the calcium-dependent inactivation of the NMDA receptor (38, 39). This indicates that α-actinin-2 also plays a regulatory role in the calmodulin/NMDA receptor interaction and does not merely anchor the receptor to the actin cytoskeleton. Further studies to establish the effects of α-actinin in $A_{2A}R$ activation of either adenylate cyclase or the ERK1/2 cascade in transfected cells as well as in neuronal primary cultures will be needed to understand the role of the cytoskeleton in adenosine neuromodulation.

In summary, a direct interaction between α-actinin and $A_{2A}R$ has been identified by the yeast two-hybrid system and confirmed by convergent techniques in transfected HEK-293 cells and in more physiological models such as cultured neurons and rat striatum. Finally, we have shown that agonist-mediated clustering and internalization of $A_{2A}R$ are mediated by its C-terminal tail and are dependent on an intact α-actinin/actin network, thus providing a functional link between this adenosine receptor and the actin cytoskeleton.

Acknowledgment—We are grateful to the personnel from Serveis Científic i Tècnics de la Universitat de Barcelona for excellent technical assistance in confocal microscopy.

REFERENCES

1. Sebastiao, A. M., and Ribeiro, J. A. (2000) *Trends Pharmacol. Sci.* **21,** 341–346
2. Fredholm, B. B., Abbracchio, M. P., Burnstock, G., Daly, J. W., Harden, T. K., Jacobson, K. A., Leff, P., and Williams, M. (1994) *Pharmacol. Rev.* **46,** 143–156
3. Marala, R. B., and Mustafa, S. J. (1993) *J. Pharmacol. Exp. Ther.* **266,** 294–300
4. Sebastiao, A. M., and Ribeiro, J. A. (1996) *Prog. Neurobiol.* (*Oxf.*) **48,** 167–189
5. Ferré, S., Fredholm, B. B., Morelli, M., Popoli, P., and Fuxe, K. (1997) *Trends Neurosci.* **20,** 482–487

6. Hettinger, B. D., Lee, A., Linden, J., and Rosin, D. L. (2001) *J. Comp. Neurol.* **431,** 331–346
7. Hillion, J., Canals, M., Torvinen, M., Casadó, V., Scott, R., Terasmaa, A., Hansson, A., Watson, S., Olah, M. E., Mallol, J., Canela, E. I., Zoli, M., Agnati, L. F., Ibáñez, C. F., Lluis, C., Franco, R., Ferré, S., and Fuxe, K. (2002) *J. Biol. Chem.* **277,** 18091–18097
8. Faulkner, G., Pallavicini, A., Comelli, A., Salamon, M., Bortoletto, G., Ievolella, C., Trevisan, S., Kojic, S., Dalla Vecchia, F., Laveder, P., Valles, G., and Lanfranchis, G. (2000) *J. Biol. Chem.* **275,** 41234–41242
9. Dunah, A. W., Wyszynski, M., Martin, D. M., Sheng, M., and Standaert, D. G. (2000) *Mol. Brain Res.* **79,** 77–87
10. Ferre, S., Karcz-Kubicha, M., Hope, B. T., Popoli, P., Burgueño, J., Gutiérrez, M. A., Casadó, V., Fuxe, K., Goldberg, S. R., Lluis, C., Franco, R., and Ciruela, F. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 11940–11945
11. Jordan, M., Schallhorn, A., and Wurm, F. M. (1996) *Nucleic Acids Res.* **24,** 596–601
12. Ciruela, F., and McIlhinney, R. A. J. (1997) *FEBS Lett.* **418,** 83–86
13. Clark, R. A. C., Gurd, J. W., Bisson, N., Tricaud, N., Molnar, E., Zamze, S. E., Dwek, R. A., McIlhinney, R. A. J., and Wing, D. R. (1998) *J. Neurochem.* **70,** 2594–2605
14. Penela, P., Alvarez-Dolado, M., Munoz, A., and Mayor, F., Jr. (2000) *Eur. J. Biochem.* **267,** 4390–4396
15. Benson, M. A., Newey, S. E., Martin-Rendon, E., Hawkes, R., and Blake, D. J. (2001) *J. Biol. Chem.* **276,** 24232–24241
16. Lefkowitz, R. J. (1998) *J. Biol. Chem.* **273,** 18677–18680
17. Mundell, S. J., Matharu, A. L., Kelly, E., and Benovic, J. L. (2000) *Biochemistry* **39,** 12828–12836
18. Ferguson, G., Watterson, K. R., and Palmer, T. M. (2002) *Biochemistry* **41,** 14748–14761
19. Peyrollier, K., Hajduch, E., Gray, A., Litherland, J., Prescott, A. R., Leslie, N. R., and Hundal, H. S. (2000) *Biochem. J.* **352,** 617–622
20. Li, M., Bermak, J. C., Wang, Z. W., and Zhou, Q. Y. (2000) *Mol. Pharmacol.* **57,** 446–452
21. Lin, R., Karpa, K., Kabbani, N., Goldman-Rakic, P., and Levenson, R. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 5258–5263
22. Hjälm, G., MacLeod, R. J., Kifor, O., Chattopadhyay, N., and Brown, E. M. (2001) *J. Biol. Chem.* **276,** 34880–34887
23. Enz, R. (2002) *FEBS Lett.* **514,** 184–188
24. Kim, M., Jiang, L., Wilson, H. L., North, R. A., and Surprenant, A. (2001) *EMBO J.* **20,** 6347–6358
25. Wyszynski, M., Lin, J., Rao, A., Nigh, E., Beggs, A. H., Craig, A. M., and Sheng, M. (1997) *Nature* **385,** 439–442
26. Hanley, J. G., Koulen, P., Bedford, F., Gordon-Weeks, P. R., and Moss, S. J. (1999) *Nature* **397,** 66–72
27. Wirkner, K., Assmann, H., Köles, L., Gerevich, Z., Franke, H., Nörenberg, W., Boehm, R., and Illes, P. (2000) *Br. J. Pharmacol.* **130,** 259–269
28. Lamaze, C., Fujimoto, L. M., Yin, H. L., and Schmid, S. L. (1997) *J. Biol. Chem.* **272,** 20332–20335
29. Munn, A. L. (2001) *Biochim. Biophys. Acta* **1535,** 236–257
30. Lunn, J. A., Wong, H., Rozengurt, E., and Walsh, J. H. (2000) *Am. J. Physiol.* **279,** C2019–C2027
31. Cooper, J. A. (1987) *J. Cell Biol.* **105,** 1473–1478
32. Miller, W. E., and Lefkowitz, R. J. (2001) *Curr. Opin. Cell Biol.* **13,** 139–145
33. Luttrell, L. M., and Lefkowitz, R. J. (2002) *J. Cell Sci.* **115,** 445–465
34. Christerson, L. B., Vanderbilt, C. A., and Cobb, M. H. (1999) *Cell Motil. Cytoskeleton* **43,** 186–198
35. Leinweber, B. D., Leavis, P. C., Grabarek, Z., Wang, C. L., and Morgan, K. G. (1999) *Biochem. J.* **344,** 117–123
36. Marti, A., Luo, Z., Cunningham, C., Ohta, Y., Hartwig, J., Stossel, T. P., Kyriakis, J. M., and Avruch, J. (1997) *J. Biol. Chem.* **272,** 2620–2628
37. Ohita, Y., Suzuki, N., Nakamura, S., Hartwig, J. H., and Stossel, T. P. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 2122–2128
38. Zhang, S., Ehlers, M. D., Bernhardt, J. P., Su, C., and Huganir, R. L. (1998) *Neuron* **21,** 443–453
39. Krupp, J. J., Vissel, B., Thomas, C. G., Heinemann, S. F., and Westbrook, G. L. (1999) *J. Neurosci.* **19,** 1165–1178

The Adenosine A_{2A} Receptor Interacts with the Actin-binding Protein α-Actinin

Javier Burgueño, Derek J. Blake, Matthew A. Benson, Caroline L. Tinsley, Christopher T. Esapa, Enric I. Canela, Petronila Penela, Josefa Mallol, Federico Mayor, Jr., Carmen Lluis, Rafael Franco and Francisco Ciruela

J. Biol. Chem. 2003, 278:37545-37552.
doi: 10.1074/jbc.M302809200 originally published online July 1, 2003
