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ACCELERATED COMMUNICATION

Cooling Evokes Redistribution of α_{2C}-Adrenoceptors from Golgi to Plasma Membrane in Transfected Human Embryonic Kidney 293 Cells

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ABSTRACT

Cold-induced vasoconstriction in cutaneous blood vessels is mediated by increased constrictor activity of vascular α_2-adrenoceptors (α_2-ARs). In mouse cutaneous arteries, α_2-AR constriction at 37°C is mediated by α_{2A}-ARs, whereas after cold exposure (28°C), α_{2C}-ARs are no longer silent and mediate the remarkable cold-induced augmentation of α_2-AR responsiveness. The goals of the present study were to develop a cell model of cutaneous thermoregulation and to determine the mechanisms underlying the thermosensitivity of α_{2C}-ARs. Human embryonic kidney 293 cells were transiently transfected with the mouse α_{2A}- or α_{2C}-AR. In cells expressing α_{2A}-ARs, UK-14,304 (5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine), an α_2-AR agonist, inhibited (10 pM) and stimulated (1–10 nM) the accumulation of cAMP evoked by forskolin. Similar responses were obtained at 37°C and 28°C. In contrast, in cells expressing α_{2C}-ARs, UK-14,304 did not affect forskolin-stimulated cAMP accumulation at 37°C but did cause a concentration-dependent inhibitory effect at 28°C. Subcellular fractionation revealed that at 37°C α_{2C}-ARs were localized predominantly to Golgi compartments, whereas α_{2A}-ARs localized predominantly to the plasma membrane. After cooling (28°C), α_{2C}-ARs relocated from Golgi compartments to the plasma membrane, whereas the α_{2A}-AR remained at the plasma membrane. Immunofluorescence microscopy confirmed that, at 37°C, α_{2A}-ARs were localized to the cell surface, whereas α_{2C}-ARs colocalized with a *trans*-Golgi marker. Cooling did not affect localization of α_{2A}-ARs, but shifted α_{2C}-ARs to the cell surface. Moderate cooling, therefore, caused a selective redistribution of α_{2C}-ARs from the Golgi compartments to the cell surface, allowing the rescue of the α_{2C}-adrenergic functional response. This mechanism may explain the role of α_2-ARs in thermoregulation of the cutaneous circulation.

α_2-Adrenoceptors (α_2-ARs) are members of the serpentine G protein-coupled family of receptors and have been classified by pharmacological and molecular cloning techniques into α_{2A}-AR, α_{2B}-AR, and α_{2C}-AR subtypes (Kobilka et al., 1987; Regan et al., 1988). These subtypes share sequence homology only in the transmembrane regions and exhibit sufficient structural heterogeneity in their intra-and extracellular domains to account for their differential regulation (Saunders and Limbird, 1999). All α_2-AR subtypes couple to G_i/G_o GTP-binding proteins to effect changes in cellular function via inhibition of adenylyl cyclase and voltage-gated Ca^{2+} channels, or activation of receptor-operated K^+ channels (Limbird, 1988). Coupling of α_2-ARs to other signal transduction pathways, such as phospholipases A_2, C, and D, and mitogen-activated protein kinase have also been described (reviewed by Saunders and Limbird, 1999). Indeed, the α_{2A}-AR subtype can both inhibit and stimulate adenylyl cyclase activity through coupling to G_i and G_s, respectively (Wade et al., 1999).

Functional α_2-ARs are not widely distributed in the vascular system; they are most prominent in small arteries and veins (Flavahan et al., 1985, 1987; Faber, 1988; Leech and Faber, 1996). α_2-AR activity is dramatically increased in the cutaneous circulation, where these receptors play an essential role in thermoregulation (Flavahan et al., 1985; Flavahan and Vanhoutte, 1986). Cold-induced vasoconstriction in the cutaneous circulation is a protective physiological response that acts to reduce

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ABBREVIATIONS: AR, adrenoceptor; HEK, human embryonic kidney; UK-14,304, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine.

loss of body heat (Vanhoutte, 1980). Cold exposure causes vasoconstriction by a reflex increase in sympathetic output of norepinephrine and by a direct local action to increase the activity of the adrenergic neurotransmitter (Vanhoutte, 1980). This latter effect is mediated by a rapid and selective augmentation of α_2-AR activity (Flavahan et al., 1985; Ekenvall et al., 1988; Faber, 1988). In cutaneous arteries of the mouse tail, α_2-AR constriction at 37°C was mediated by α_{2A}-ARs, with no apparent role for α_{2C}-ARs (Chotani et al., 2000). However, upon cold exposure (28°C), α_{2C}-ARs were no longer silent and mediated the remarkable cold-induced augmentation of α_2-AR responsiveness. These data implicate the α_{2C}-AR as a putative thermosensor in the vessel wall and are consistent with the original notion of the α_{2C}-AR as a "silent receptor" (MacDonald et al., 1997). Indeed, α_{2C}-ARs are localized to intracellular compartments in rat fibroblasts at 37°C (Daunt et al., 1997).

The goal of the present study was to generate a cell model of cutaneous vasoconstriction using HEK293 cells transfected with α_{2A}-ARs or α_{2C}-ARs and to determine the mechanism underlying the thermosensitivity of α_{2C}-ARs.

Experimental Procedures

Cell Culture and Transfection. Human embryonic kidney 293 cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's modifed Eagle's medium containing penicillin/streptomycin, supplemented with fetal bovine serum (10%) at 37°C in 5% CO_2. Murine α_2-ARs were subcloned into the pCDNA3 expression vector (gift from B. Kobilka, Stanford University, Stanford, CA), and cells were transfected when 40% confluent with either pCDNA3-α_{2A}-AR or pCDNA3-α_{2C}AR using the FuGENE 6 transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN). Equal expression of receptor subtypes was confirmed by immunoblot analyses and densitometry (major band at 70 kDa: α_{2A}-AR, 1356.3 ± 80.5; α_{2C}-AR, 1420 ± 48.0, arbitrary units, $P > 0.05$, $n = 3$).

Measurement of Cellular Cyclic AMP Accumulation. Cellular accumulation of cyclic AMP was measured by radioimmunoassay using a commercial kit (Biomedical Technologies, Inc., Stoughton, MA). Briefly, transfected 293 cells were grown to 80% confluence in 12-well plates at 37°C. On the day of the study, cells were incubated in Dulbecco's modifed Eagle's medium at 37°C or 28°C for 1 h. The cells were pretreated with 3-isobutyl-1-methylxanthine (0.225 mM, 30 min) at their respective temperatures, before exposure to the α_2-AR agonist UK-14,304 (10 pM-10 nM; one concentration per well) for 1 min, before adding forskolin (3 μM) for an additional 5 min. After these treatments, the cells were placed on ice and washed twice with ice-cold phosphate-buffered saline (with Ca^{2+}/Mg^{2+}), and lysed with 10 mM HCl in EtOH for 30 min. Samples were centrifuged and the supernatant was collected for measurement of intracellular cAMP production using a radioimmunoassay ^{125}I-cyclic AMP kit.

Subcellular Fractionation. Transfected 293 cells were harvested by gentle scraping in buffer A (2 ml; 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 2 mM $MgCl_2$) containing anti-proteases (15.7 μg/ml each chymostatin, antipain, and pepstatin; 57.7 μg/ml leupeptin, and 250 μg/ml 4-(2-aminoethyl)benzenesulfonyl fluoride). Cell homogenates were prepared as described previously (Hurt et al., 2000), and centrifuged (1000g, 5 min, 4°C) to remove cell debris and nuclei. Sucrose (2 M) was added to the postnuclear supernatant to yield a final concentration of sucrose (0.2 M). Each sample (30 μg of protein) was layered over a discontinuous sucrose gradient (0.5, 0.9, 1.2, 1.35, 1.5, and 2 M, each at 1.5 ml) and centrifuged in a SW40Ti rotor (28,000 rpm, 16 h, 4°C; Beckman Coulter, Fullerton, CA). Ten fractions (1 ml) were collected drop-wise from the bottom of the tube by piercing with a needle (26$G_{5/8}$). Proteins were precipitated by the addition of trichloroacetic acid (100%, 100 μl) and freezing (−20°C) overnight. After protein extraction with ether/ethanol (1:1), the fractions were mixed with SDS sample buffer, resolved by SDS-polyacrylamide gel electrophoresis on 7 or 10% gels, and bands were quantified by densitometry (Personal Densitometer; Molecular Dynamics, Sunnyvale, CA).

Indirect Immunofluorescence Microscopy. 293 cells (50,000 cells) were plated on poly(D-lysine)-coated 25-mm coverslips. Twenty-four hours after attachment, cells were cotransfected with plasmid DNA encoding either the α_{2A}-AR or α_{2C}-AR (0.5 μg of DNA per coverslip) and pECFP-Golgi vector (1 μg per coverslip) for 48 h, and then treated as indicated under *Results*. pECFP-Golgi vector (CLONTECH, Palo Alto, CA) encodes a fusion protein comprising ECFP and the amino-terminal 81 amino acids of human β 1,4-galactosyltransferase, which targets the fusion protein to the *trans*-medial Golgi. Cells were fixed in paraformaldehyde (3%, 4°C, 30 min), and processed for microscopic imaging as detailed previously (Moore et al., 1995). In certain experiments, cells were permeabilized using Triton X-100 (0.2%). α_{2A}- and α_{2C}-ARs were tagged with the hemagglutinin epitope at their extracellular amino termini, and comparison of images obtained from permeabilized and nonpermeabilized cells allowed a distinction between intracellular and cell surface receptor staining. The concentrations of antibodies used were: 0.2 μg/100 μl mHA.11 and 0.15 μg/100 μl goat anti-mouse Cy3. Coverslips were mounted using the Slowfade Light Antifade kit (Molecular Probes, Eugene, OR) on precleaned glass micro slides (Corning Glass Works, Corning, NY) and viewed using a Eclipse E800 fluorescent microscope (Nikon, Tokyo, Japan). Images were collected using Metamorph Software (Universal Imaging Corporation, Downingtown, PA).

Materials. mHA.11 antibody against the HA-epitope was from Berkeley Antibody Co. (Berkeley, CA) and goat anti-mouse Cy3 was from Molecular Probes (Eugene, OR). Cyclic AMP radioimmunoassay kits were purchased from Biochemical Technologies Inc. (Stoughton, MA). Forskolin and 3-isobutyl-1-methylxanthine were from Sigma Chemical Co. (St Louis, MO), UK-14,304 was obtained from Sigma/RBI (Natick, MA), and mastoparan was purchased from Calbiochem (San Diego, CA). Protein assays were performed using the Bradford method (Bio-Rad Laboratories, Hercules, CA).

Statistics. Statistical evaluation of the data was performed by Student's t test for either paired or unpaired observations. When more than two means were compared, analysis of variance was used. If a significant F value was found, Scheffé's test for multiple comparisons was employed to identify differences among groups. Values were considered to be statistically different when P was less than 0.05.

Results

α_2-AR-Signaling. Forskolin-induced accumulation of cAMP was not statistically different under warm (37°C) and cold (28°C) conditions in 293 cells expressing α_{2A}-ARs or α_{2C}-ARs, or in mock transfected cells (Fig. 1). In mock transfected cells, the α_2-AR agonist UK-14,304 failed to alter the levels of cAMP stimulated by forskolin under either warm or cold conditions (data not shown). In 293 cells expressing α_{2A}-ARs, UK-14,304 inhibited (10 pM) and stimulated (1–10 nM) the accumulation of cAMP evoked by forskolin, and neither effect was altered by lowering the temperature to 28°C (Fig. 1A). In contrast, in cells expressing α_{2C}-ARs, UK-14,304 (10 pM-10 nM) did not significantly affect the accumulation of cAMP stimulated by forskolin (3 μM) at 37°C but did cause a concentration-dependent reduction in cAMP levels in cells incubated at 28°C (Fig. 1B). Mastoparan (0.1 μM–0.1 mM), a direct activator of the G_i-protein, caused similar reductions in cAMP accumulation evoked by forskolin at warm and cold temperatures in mock-transfected 293 cells (Fig. 1C).

Subcellular Fractionation. Golgi fractions were identified with an anti-β-COP monoclonal antibody, and plasma membrane fractions with an anti-Na^+-K^+-ATPase α-subunit monoclonal antibody. The discontinuous fractionation protocol separated fractions enriched with Golgi membranes (fraction 3, interphase between 0.9 and 0.5 M sucrose) from those fractions enriched with plasma membranes (fraction 6, interphase between 1.2 and 1.35 M sucrose) (Fig. 2A). Cooling of cells for 1 h (28°C) did not affect the distribution of Golgi- or plasma membrane-enriched fractions.

At 37°C, expression of α_{2A}-ARs (70-kDa species) was predominantly at the plasma membrane (fraction 6), and this pattern was not influenced by cooling to 28°C (Fig. 2A). Therefore, the plasma membrane: Golgi ratio for α_{2A}-ARs was greater than 1 and was not significantly different between warm and cold conditions (Fig. 2B). In contrast, at 37°C, α_{2C}-ARs (70-kDa species) were expressed robustly in fraction 3 (Golgi) but to a much lesser extent in fraction 6 (plasma membrane) (Fig. 2A). Cooling from 37°C to 28°C, however, resulted in marked diminution of α_{2C}-AR expression in fraction 3 (Golgi), with concomitant increased receptor expression in fraction 6 (plasma membrane) (Fig. 2A). Therefore, the plasma membrane/Golgi ratio for α_{2C}-ARs was significantly increased upon cooling (37°C versus 28°C; 0.6 ± 0.01 versus 2.7 ± 0.5; $P < 0.05$, $n = 3$) (Fig. 2B).

Immunofluorescence. The pECFP-Golgi vector gave a characteristic perinuclear staining appearance in both nonpermeabilized and permeabilized cells (Fig. 3, B and E, and Fig. 4, B and E). Cooling of the cells did not affect the cellular distribution of the pECFP-Golgi vector (Fig. 3, H and K, and Fig. 4, H and K).

At 37°C, α_{2A}-ARs were localized to the cell surface and could be visualized in both nonpermeabilized (Fig. 3A) and permeabilized cells (Fig. 3D). Cooling from 37°C to 28°C caused no significant changes in the surface staining pattern of the α_{2A}-ARs (Fig. 3, G and J), although an intense perinu-

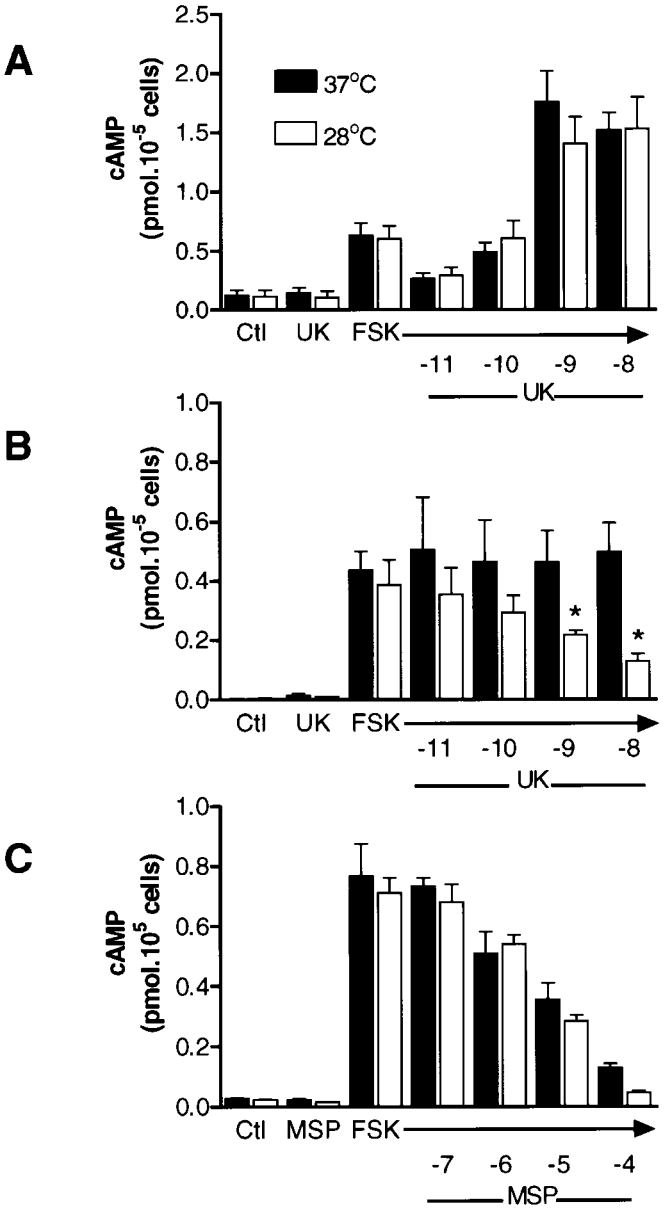

Fig. 1. Effect of cooling from 37°C to 28°C on inhibition of forskolin-stimulated cellular accumulation of cyclic AMP evoked by UK-14,304 or mastoparan in 293 cells expressing α_2-ARs. Cells were either transiently transfected with plasmid encoding α_{2A}-ARs (A) or α_{2C}-ARs (B) or were mock transfected (C). Cells were incubated at 37°C or 28°C for 60 min and were exposed to UK-14,304 or mastoparan (single concentration for 1 min) at the respective temperature, followed by forskolin (3 μM, 5 min). Cellular accumulation of cyclic AMP was assayed as described under *Experimental Procedures*. Data are expressed as mean ± S.E.M. *, $P <$ 0.05 compared with 37°C; $n = 4–6$.

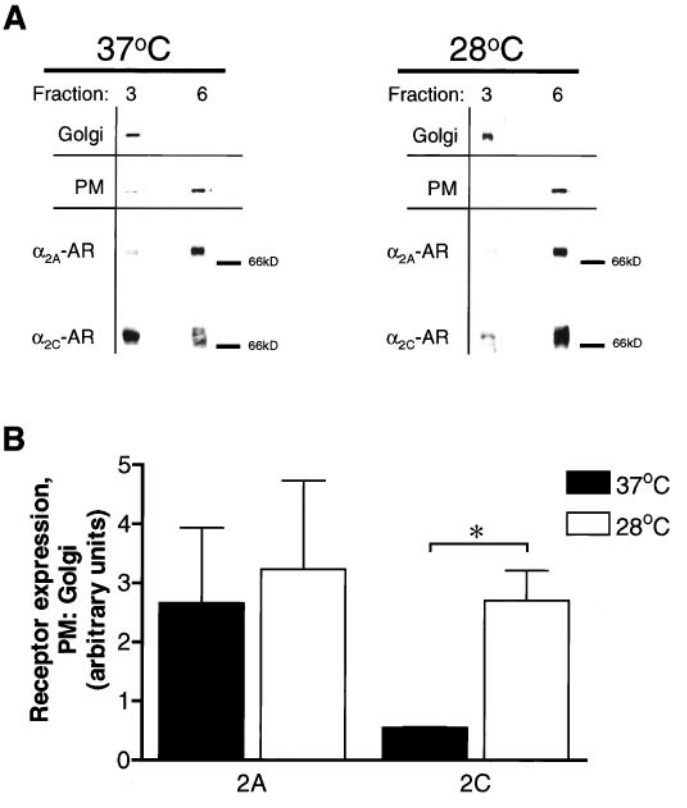

Fig. 2. Effect of cooling on subcellular distribution of α_{2A}-ARs and α_{2C}-ARs in transiently transfected 293 cells. Cells expressing either α_{2A}-ARs or α_{2C}-ARs were incubated at 37°C or 28°C for 1 h, before harvesting and processing for subcellular fractionation as described under *Experimental Procedures*. Golgi-enriched fractions were characterized using a monoclonal anti-βCOP antibody, and the plasma membrane was characterized using a monoclonal antibody against the α-subunit of the Na^+-K^+-ATPase. A, representative immunoblots from two to three similar experiments. B, Immunoblots were quantified by densitometry and receptor expression presented as the plasma membrane/Golgi ratio. Data are expressed as mean ± S.E.M. *, $P < 0.05$.

clear staining signal was also observed in permeabilized cells (Fig. 3J), which colocalized with pECFP-Golgi vector (Fig. 3L). The α_{2A}-AR cell surface staining was distinct from that of the pECFP-Golgi vector at both 37°C and 28°C (Fig. 3, C, F, I, and L).

At 37°C, α_{2C}-ARs were not localized to the cell surface, demonstrating a very weak staining signal in nonpermeabilized cells (Fig. 4A), and a perinuclear staining pattern in permeabilized cells (Fig. 4D), which colocalized with the pECFP-Golgi vector (Fig. 4F). Contrariwise, α_{2C}-ARs showed a distinct re-localization in response to cooling. In permeabilized cells, α_{2C}-ARs localized to the Golgi as well as the plasma membrane (Fig. 4L). This change in localization was most striking in nonpermeabilized cells, in which receptor staining at the cell surface (Fig. 4G) was clearly distinct from the pECFP Golgi vector staining (Fig. 4I).

Discussion

Constriction of cutaneous blood vessels in response to cooling is a protective physiological response that acts to reduce loss of body heat (Vanhoutte, 1980). Cooling effects a reflex increase in sympathetic output of norepinephrine and a direct local increase in the activity of the adrenergic neurotransmitter at smooth muscle cells (Vanhoutte, 1980). The direct constrictor response to cold is mediated at the cellular level by the rapid and selective augmentation of α_2-AR activity (Flavahan et al., 1985; Ekenvall et al., 1988; Faber, 1988). Specifically, it has been shown that increased activity of α_{2C}-AR function mediates the augmented vasoconstriction to cooling in mouse arterial microvessels (Chotani et al., 2000). Thus, the α_{2C}-AR may act as a putative thermosensor in the vessel wall. The mechanisms by which α_{2C}-ARs become pharmacologically active by cold exposure are not understood and formed the focus of this study. To this end, we developed a cell model in which to characterize the thermosensitivity of the α_{2C}-AR. Indeed, transfection of HEK293 cells with mouse α_{2C}-ARs, but not α_{2A}-ARs, engendered thermosensitivity in these cells. We used functional, biochemical, and immunocytochemical techniques to demonstrate that moderate cooling evoked a selective recompartmentalization of α_{2C}-ARs from the Golgi network to the plasma membrane. These changes in the steady-state distribution of α_{2C}-ARs uncovered a functional response to agonist stimulation.

Cooling from 37°C to 28°C for brief intervals is sufficient to uncover α_{2C}-AR-mediated vasoconstriction (Chotani et al.,

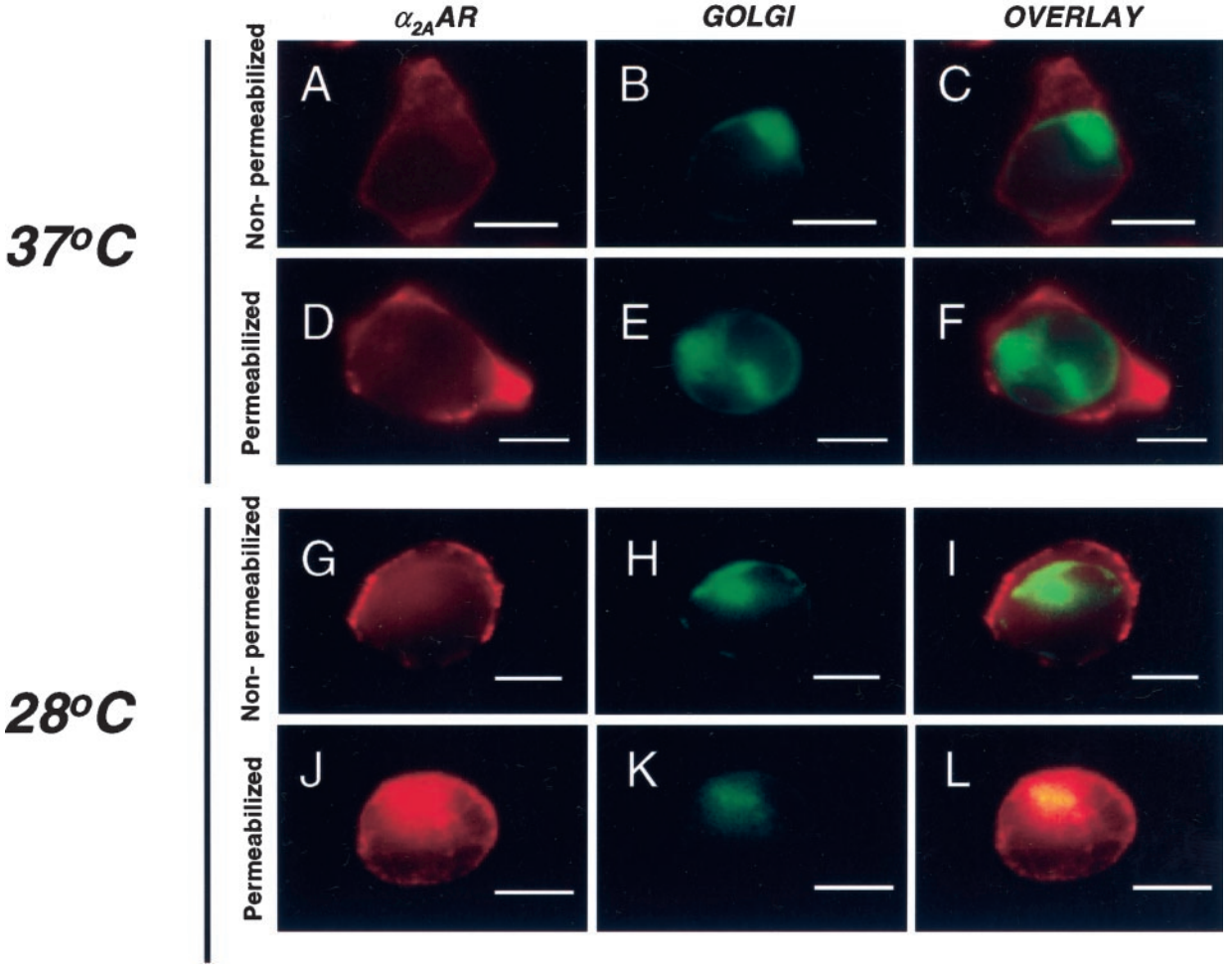

Fig. 3. Effect of cooling on localization of α_{2A}-ARs in nonpermeabilized and permeabilized HEK293 cells as visualized by indirect immunofluorescence microscopy. 293 cells were transiently transfected with HA-epitope tagged α_{2A}-ARs, fixed, and processed as described under *Experimental Procedures*. α_{2A}-ARs were detected using the mHA.11 monoclonal antibody and goat anti-mouse Cy3 secondary antibody (A, D, G, and J). Golgi compartments were visualized using the pECFP-Golgi vector (B, E, H, and K). Overlays of images are shown in C, F, I, and L. Images are representative of three similar experiments. Scale bar is 10 μm.

2000). Thus, a similar cooling regimen (28°C for 1 h) was employed to study the effects of lowering temperature on α_2-AR responsiveness in a transfected cell system. In 293 cells transiently expressing α_{2C}-ARs, the α_2-AR agonist UK-14,304 failed to inhibit the accumulation of cAMP stimulated by forskolin at all concentrations studied at 37°C. Cooling to 28°C, however, uncovered a marked concentration-dependent inhibitory capacity for UK-14,304. The influence of cooling was selective for the α_{2C}-AR, because the responses to UK-14,304 in 293 cells expressing α_{2A}-ARs were not affected by changes in temperature. α_{2A}-ARs and the α_{2C}-ARs both inhibit adenylyl cyclase via activation of G_i-proteins, although α_{2A}-ARs can also couple to G_s (Eason et al., 1992) generating biphasic concentration-effect curves to agonist stimulation (Wade et al., 1999), as observed in the present study. A lack of effect of cold on responses to α_{2A}-AR activation suggests that moderate cooling does not markedly affect signaling events downstream of α_2-ARs. Indeed, responses to direct activation of adenylyl cyclase by forskolin or to direct activation of G_i-proteins by mastoparan (Higashijima et al., 1990) were not affected by these moderate changes in temperature. These data implicate the α_{2C}-AR as a specific thermosensor in transfected HEK293 cells.

Subcellular fractionation of 293 cells expressing α_{2C}ARs revealed that the α_{2C}-ARs were localized predominantly to intracellular Golgi compartments and that, upon cooling, this receptor subtype translocated to the plasma membrane. In contrast, α_{2A}-ARs were located predominantly at the plasma membranes at warm and cold temperatures. Both receptor subtypes were expressed as 70-kDa species, which represents the glycosylated form of the receptors. Deglycosylation of receptors with PNGase F in a transfected cell system converted this high-molecular-mass receptor to a species of smaller mass (40–45 kDa) (Chotani et al., 2000). Although the role of glycosylation in the regulation of α_2-AR function has not been established, the 70 kDa species may represent the functional form of the receptor. Indeed, a direct correlation between the level of expression of the glycosylated receptor species and α_2-AR function was observed in mouse tail arterioles (Chotani et al., 2000).

The fundamental observation that α_{2C}-ARs are relocated to the cell periphery upon moderate cooling of cells was confirmed by visualization of epitope-tagged receptors by immunofluorescence microscopy. At 37°C, substantial colocalization of α_{2C}-ARs with the *trans*-Golgi marker pECFP-Golgi vector was observed. Previous studies, however, have reported that the majority of α_{2C}-ARs expressed in rat fibroblasts at 37°C are confined to the endoplasmic reticulum and

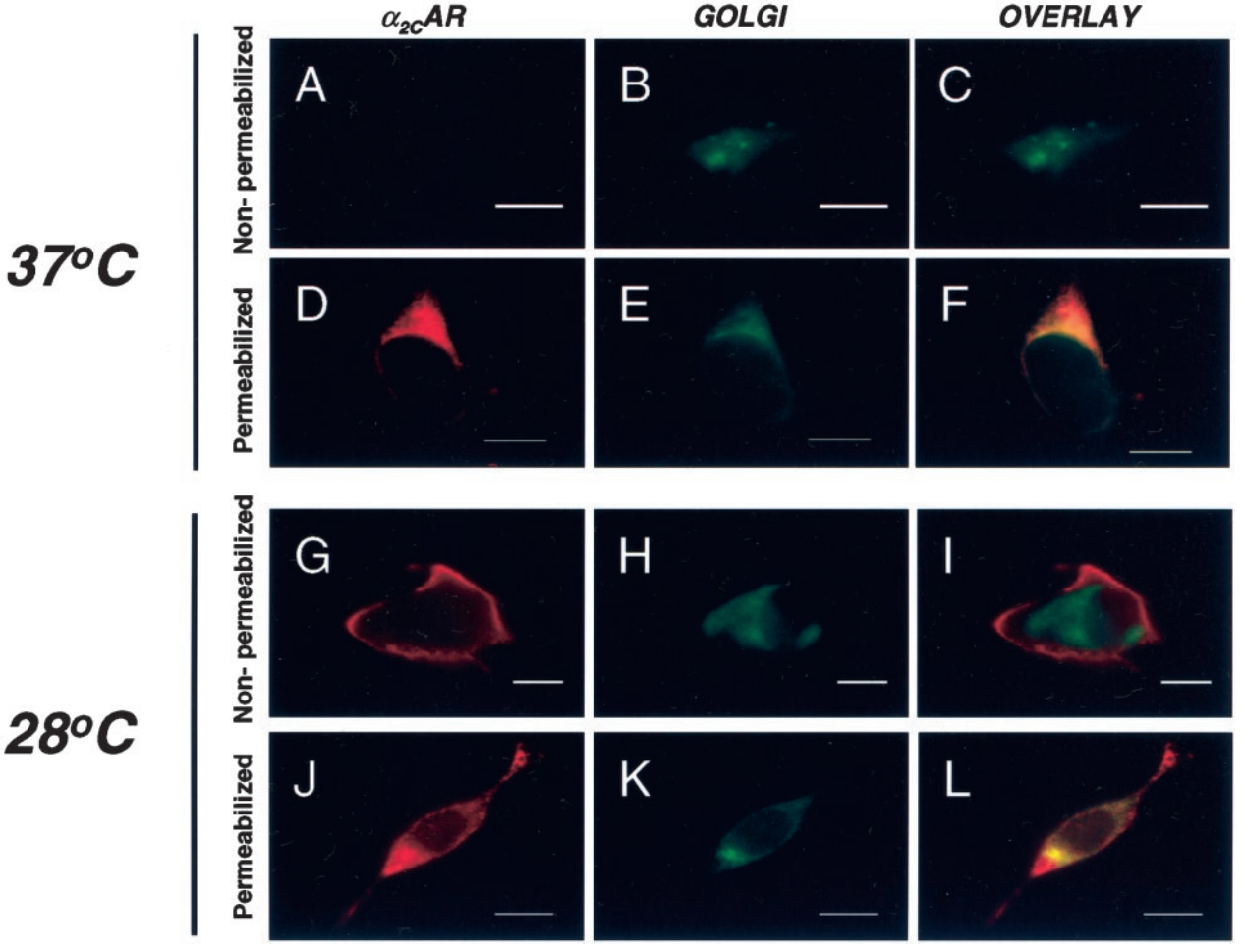

Fig. 4. Effect of cooling on localization of α_{2C}-ARs as visualized by indirect immunofluorescence microscopy. 293 cells were transiently transfected with HA-epitope tagged α_{2C}-ARs and were then fixed and processed as described under *Experimental Procedures*. α_{2C}-ARs were detected using the mHA.11 monoclonal antibody and goat anti-mouse Cy3 secondary antibody (A, D, G, and J). Golgi compartments were visualized using the pECFP-Golgi vector (B, E, H, and K). Overlays of panels are shown in C, F, I, and L. Images are representative of three similar experiments. Scale bar is 10 μm.

cis-medial Golgi compartments (Daunt et al., 1997). This discrepancy may be related to the cell system chosen for study, because the intracellular localization of α_{2C}-ARs is highly cell-type dependent (Hurt et al., 2000). For example, α_{2C}-ARs are retained in intracellular compartments in HEK293 cells (this study), COS-7 cells, NRK cells, Madin-Darby canine kidney cells, and rat1 fibroblasts but are localized to the cell surface in PC12 and AtT20 neuroendocrine cells (Hurt et al., 2000). Comparative studies among these cell types may provide clues as to the mechanism by which α_{2C}-ARs are differentially distributed and regulated and whether such mechanisms are initiated by cooling.

Redistribution of α_{2C}-ARs from the Golgi to the cell surface did not result from loss of integrity of the cellular compartments. The subcellular fractionation protocol provided a clean and reproducible separation of fractions enriched in Golgi or plasma membranes during both warm and cool conditions. Furthermore, immunofluorescence microscopy showed that the staining pattern of the Golgi marker pECFP-Golgi was unaffected by cooling, suggesting that the integrity of the Golgi remained intact.

Spatial relocation of α_{2C}-ARs to the cell surface during cooling rescued a functional α_{2C}-AR response. A large pool of functional α_{2C}-ARs, however, is found in the endoplasmic reticulum of NRK cells, as determined by radioligand binding assay (Hurt et al., 2000). The existence of an intracellular store of functional α_{2C}-ARs may facilitate the rapid cooling-induced translocation of receptors to the cell surface by negating the need for further biochemical or structural modification. Certainly, the size of the receptor protein that undergoes recompartmentalization in response to cooling is the same in both Golgi and plasma membrane fractions. Thus, cooling may target post-translational events in the α_{2C}-AR biosynthetic pathway. Indeed, inhibition of receptor translation with cycloheximide does not selectively impair the expression of α_{2C}-ARs in HEK293 cells or the α_{2C}-AR-mediated vasoconstriction to cooling (unpublished data). A scenario may be imagined, therefore, in which mature and functional receptors are stored in the Golgi compartments in readiness for an appropriate translocation stimulus. Interestingly, a similar mechanism has been described for the mutant cystic fibrosis transmembrane conductance regulator, ΔF508 CFTR, which is retained in the endoplasmic reticulum-Golgi compartments but undergoes spatial and functional rescue by cooling or addition of small solvent chaperones (Denning et al., 1992; Brown et al., 1996). The possibility exists that both the α_{2C}-AR and cystic fibrosis transmembrane conductance regulator share the same regulatory pathway in response to cooling.

The selective recruitment of α_{2C}-ARs to the cell surface during cooling reported here may help explain the augmented vasoconstrictor response to α_2-AR stimulation observed in the cutaneous microcirculation (Chotani et al., 2000). Moreover, these findings highlight the α_{2C}-AR as a potential target for therapeutic intervention in vascular disorders such as Raynaud's phenomenon, which is associated with a heightened cold-induced α_2-AR vasoconstriction (Freedman et al., 1995). In conclusion, this study demonstrates that moderate cooling causes selective recompartmentalization of α_{2C}-ARs from the Golgi network to the plasma membrane, where these receptors are responsive to agonist stimulation.

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