Richman, Jeremy G., and John W. Regan. \(\alpha_2\)-Adrenergic receptors increase cell migration and decrease F-actin labeling in rat aortic smooth muscle cells. Am. J. Physiol. 274 (Cell Physiol. 43): C654–C662, 1998.—Vascular wound healing and such pathologies as atherosclerosis and restenosis are characterized by migration and proliferation of the smooth muscle cells of the media after denudation of the intima. To explore possible roles that \(\alpha_2\)-adrenergic receptors (\(\alpha_2\)-ARs) might have in these cellular responses, we characterized the \(\alpha_2\)-ARs present in explant-derived cultures of rat aortic smooth muscle (RASM) cells. The results of immunofluorescence microscopy and reverse transcription followed by the polymerase chain reaction indicated that all three \(\alpha_2\)-AR subtypes (\(\alpha_2A\), \(\alpha_2B\), and \(\alpha_2C\)) were initially present. Mitogen-activated protein kinase activity in the RASM cells was stimulated fivefold over basal by the \(\alpha_2A\)-selective agonist dexmedetomidine (Dex) and was blocked by coincubation with the \(\alpha_2A\)-selective antagonist rauwolscine (RW) or by preincubation of the cells with the G\_G\_protein inhibitor pertussis toxin. \(\alpha_2\)-AR activation by Dex also produced a marked decrease in F-actin labeling, which again was prevented by coincubation with RW. The evidence clearly reveals the presence of functional \(\alpha_2\)-ARs in RASM cells. The involvement of \(\alpha_2\)-AR activation with cytoskeletal changes and cell migration is novel and indicates a potential role of these receptors in vascular wound healing and pathogenesis.

atherosclerosis; G protein-coupled receptor; vascular wound healing; chemokinesis

**MATERIALS AND METHODS**

Materials. Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin (Pen/Strep), fetal bovine serum (FBS), and 1× trypsin with EDTA (0.05 and 0.02% wt/vol) were purchased from Gibco BRL (Grand Island, NY). Fluorescein isothiocyanate (FITC)-labeled rabbit anti-chicken and Cy5-labeled rabbit anti-chicken immunoglobulin were from Jackson Immunoresearch Laboratories (West Grove, PA). Cell culture dishes were from Falcon (Lincoln Park, NJ).

RASM cell isolation and culture. RASM cells were prepared as adapted from Ross (26). Male rats (175–185 g, Harlan Sprague Dawley) were killed by CO\_2 asphyxiation, and the thoracic aorta was removed along with the aortic arch. Vessels were stripped of adventitia and cut longitudinally, and the endothelium was removed by scraping with scissors. Tissue was cut into pieces (3 mm\(^2\)) and placed intima side down in six-well culture plates (3–5 pieces/well). DMEM containing Pen/Strep (50 U/ml and 50 mg/ml, respectively) and 10% FBS was added, and the plates were placed in a
humidified incubator at 37°C with 5% CO2-95% air. After 7–10 days, the cells began to migrate out from the tissue sections, reaching confluence in ~20 days. Upon confluence, the cells were detached with 0.05% trypsin-0.02% EDTA and seeded on 100-mm plates at a density of 3 × 10^6 cells/plate (passage 1). Cells were then split 1:3 approximately every 3 days and were identified by the passage number and animal of origin (e.g., passage 2 from animal F would be labeled P2,F).

Antibody production and characterization. Antibodies were raised in chickens to glutathione-S-transferase (GST) fusion proteins containing unique extracellular domains of each of the α2-AR subtypes. Methods for the preparation of the fusion proteins and antibody purification were as previously described (13, 30). The primers used for the initial polymerase chain reactions (PCR) and the regions amplified for each of the α2-AR subtypes were as follows: α2A amino terminus (amino acids 4–26), α2A sense primer 5'-CCCCATGGATCCCC-TGCAGCCGGACCGGCG-3', α2A antisense primer 5'-CA-GGGAGAATTCGTGCAGCCGGACCGGCG-3'; α2B second extracellular loop (amino acids 150–177), α2B sense primer 5'-CCGCCCGGATCTACAAGGGCGACCAGGGC-3', α2B antisense primer 5'-GAAGAAGAGAATTCGGAGGCGAGATT-GTACCA-3'; α2C amino terminus (amino acids 3–45), α2C sense primer 5'-ACCATGGGATCCCGCGCTGGCGGCGGCGGCG-3', α2C antisense primer 5'-GCGCGCCGAATTTCTCGCCGC-GCGGCCGC-3'. PCR products were ligated in frame behind its corresponding fusion protein but not with GST or the other fusion proteins.

Immunofluorescence microscopy. Cells were passaged onto glass coverslips, and after 24–48 h, they were rinsed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde in PBS. After an incubation with NaN3 (1 mg/ml), the cells were permeabilized in 0.05% saponin in PBS containing 10% goat serum and were incubated with the primary antibody for 2–4 h at room temperature. Cells were then washed (3 times, 15 min each) in PBS under gentle agitation. Incubation with the secondary antibody was for 40 min at room temperature in the same buffer in the dark. Cells were washed in PBS (3 times, 10 min each) with gentle agitation and mounted onto glass slides with 10 µl of p-phenylenediamine, using Cytoseal (Stephens Scientific, Riverdale, NJ). For F-actin labeling, cells were incubated with Texas red isothiocyanate (TRITC)-labeled phalloidin (Pierce) for 2 h at room temperature, washed (3 times, 5 min each) with PBS, and mounted as before.

For visualization of fluorescent labeling, epifluorescent microscopy was performed using an Olympus BH-2 microscope with a ×60 oil objective (1.4 numerical aperture), with a 100-W mercury bulb, through a dichroic FITC filter cube. Alternatively, samples were visualized using a Leica-TCS confocal microscope (Deerfield, IL). All conditions were performed in duplicate or triplicate and replicated at least three times. A minimum of five fields was randomly sampled per coverslip. All images chosen for figures were taken under identical conditions (exposure time, voltage intensity, pinhole size, etc.) and were representative of each condition.

Reverse transcriptase-PCR. Total RNA was isolated from confluent plates (10 cm) of cultured RASM cells using TriZol (GIBCO BRL) according to the manufacturer's instructions. The final pellets were resuspended in 100 µl of water, and the concentration and relative purity of the RNA was determined by spectroscopy (260/280 nm OD). Reverse transcriptase reactions were done using SuperScript II (GIBCO BRL) according to the manufacturer's specifications, using 200 ng of random primers mix (hexamers), 500 ng oligodT(12–18-mer), and 3 µg of total RNA per reaction. To rule out amplification from genomic DNA, reverse transcriptase (RT) reactions were done in parallel with a preincubation with either ribonuclease (RNase) or RNase inhibitor for 1 h at 37°C. PCR reactions were performed using 2 µl of the RT reaction as template. The reactions consisted of an initial hot start at 96°C and 40 cycles of 96°C for 1 min, 55°C for 2 min, and 72°C for 3 min followed by a final incubation at 72°C for 10 min. Products were separated on 1.4% agarose gels and stained with ethidium bromide. α2-AR-specific primers were chosen based on their uniqueness (determined using the Basic Local Alignment Search Tool at the National Center for Biotechnology Information) and analysis via the computer program Oligo (National Biosciences, Plymouth, MN).

MAP kinase assay. Mitogen-activated protein (MAP) kinase assays were performed as described by Burkey and Regan (4) with the exception that immunoprecipitation was performed using a monoclonal anti-ERK 1 (p44) MAP kinase antibody or a combination of both polyclonal anti-ERK 1 and ERK 2 MAP kinase antibodies (GIBCO BRL and Santa Cruz Biotechnology, respectively). Cells were grown to confluence and then cultured overnight in serum-free DMEM containing insulin, transferrin, and selenium (ITS, GIBCO BRL). Cells were then incubated in the same media containing drugs for 5–10 min at 37°C. Alternatively, some cells were incubated with drug after a 4-h preincubation with 150 ng/ml pertussis toxin (PTx). Cells were washed with PBS (4°C) and scraped into lysis buffer (50 mM β-glycerophosphate, 1 mM ethylene glycol-bis-[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid, 2 mM MgCl2, 100 µM NaVO3, 0.5% Triton X-100, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20 µM pepstatin, and 20 µM leupeptin at pH 7.2 and 4°C). Cell debris was sedimented by centrifugation at 12,000 g for 15 min at 4°C, and 100-µg aliquots of the lysate were used for the MAP kinase assay. Anti-MAP kinase antibodies (2 µg ERK 1/ERK 2, Santa Cruz Biotechnology) were added to the lysate and incubated overnight with rotation at 4°C. Protein G agarose (20 µl; Calbiochem, La Jolla, CA) was then added, incubated for 2 h at 4°C and spun at 12,000 g. The pellet was washed twice in lysis buffer and one time in kinase buffer [lysis buffer containing 12.5 µg/ml MgCl2 and 25 µg/ml...
protein kinase A inhibitor (Sigma). The washed pellet was resuspended in kinase buffer with 1 mg/ml myelin basic protein (GIBCO BRL) and 5 µM \[^{32}P\]ATP (NEN, Boston, MA) in a final volume of 40 µl. Reactions proceeded for 15 min at 37°C and were terminated with the addition of 10 µl of 25% (wt/vol) trichloroacetic acid. Aliquots (20 µl) were filtered onto Whatman GF/B filters through a Brandel cell harvester (Gaithersburg, MD) and washed with 75 mM phosphoric acid (10 ml each). Radioactivity was determined by liquid scintillation counting. Multiple induction was calculated by dividing the sample values by the unstimulated control values (after subtracting background, e.g., absence of lysate).

Proliferation assay. \[^{3}H\]Thymidine incorporation assays were performed as a marker of cell proliferation. Initially, primary culture aortic smooth muscle cells were plated at a density of 150 cells/mm\(^2\) in a 96-well plate (Corning, Corning, NY) and grown 14 h in DMEM(−), 95% air-5% CO\(_2\). The next day, cells were washed with PBS (37°C) and incubated for 24-48 h in DMEM without FBS (length of time was a function of the assay, and a number of times were examined; generally, a 24-h serum starvation period was applied). Cells were then exposed to DMEM alone or containing 2% FBS (positive control), agonist (1 µM dexmedetomidine (Dex), or agonist plus antagonist (1 µM Dex + 1 µM rauwolscine (RW)) and 0.1 µCi \[^{3}H\]Thymidine (Amersham, Arlington Heights, IL). Next, cells were incubated overnight, lysed in H\(_2\)O, and harvested onto Whatman GF/B filters, using a Brandel cell harvester. The filters were then washed repeatedly (~10 ml/well) with ice-cold H\(_2\)O, dried in an oven (55°C), and counted in Safety-Solve (Research Products International). A number of parameters were examined, including incubating with agonist in the presence of ITS, 0.1% FBS, 2% FBS, and lower cell densities (1×10\(^4\) and 0.5×10\(^4\) cells/well).

In addition, the assays were performed on cells seeded at 300% confluence (a density of 2,000 cells/mm\(^2\)) in 12-well plates containing inserts designed to yield a 3×20-mm rectangular cell reservoir to mimic the conditions of cell migration assays. Cells were allowed to adhere for 2.5 h in DMEM containing 10% FBS and Pen/Strep, after which time the inserts were removed and the cell strips were washed and incubated overnight in DMEM containing ITS and Pen/Strep. Cells were next incubated in DMEM alone, containing 2% FBS (positive control) or with agonist (1 µM Dex), and 0.1 µCi \[^{3}H\]Thymidine was added to each for 48 h. Cells were then lysed in H\(_2\)O and harvested onto Whatman GF/B filters, using a Brandel cell harvester. The filters were washed repeatedly (~10 ml/well) with ice-cold H\(_2\)O, dried in an oven (55°C), and counted in Safety-Solve (Research Products International).

Fig. 1. Characterization of \(\alpha_2\)-adrenergic receptor (AR) subtype-selective polyclonal antibodies. COS-7 cells were transiently transfected with the \(\alpha_2A\)-ARs (A–C), \(\alpha_2B\)-ARs (D–F), or \(\alpha_2C\)-ARs (G–I). Immunofluorescent microscopy was then done as described in MATERIALS AND METHODS. A, D, and G show fluorescence of cells after incubation with anti-\(\alpha_2A\)-NH\(_2\) polyclonal antibodies. B, E, and H show fluorescence of cells after incubation with anti-\(\alpha_2B\)-2ECL polyclonal antibodies. C, F, and I show fluorescence of cells after incubation with anti-\(\alpha_2C\)-NH\(_2\) polyclonal antibodies. Secondary antibody was a fluorescein isothiocyanate-rabbit anti-chicken immunoglobulin. Images are representative of at least 3 coverslips and 5 random fields/coverslip. Experiments have been repeated at least 4 times with similar results.
α₂-adrenergic receptors in RASM cells

Cell migration assay. The effects of drugs on the migration of RASM cells was determined using the linear migration assay described by Hoying and Williams (12; see also Ref. 28). Cells were seeded at 300% confluence (~2,000 cells/mm²) in 12-well plates containing inserts designed to yield a 3 × 20-mm rectangular cell reservoir from which cell migration could occur. In the present study, inserts were made of Teflon. We found that inserts made out of Delrin, as used previously, were toxic to RASM cells. Cells were allowed to adhere for 2.5 h in DMEM containing 10% FBS and Pen/Strep, after which time the inserts were removed and the cell strips were washed and incubated overnight in DMEM containing ITS and Pen/Strep. The cells were then incubated with or without drugs for 48 h. The media was changed every 24 h, and drug was readministered to the appropriate wells. The cells were then fixed for 30 min in PBS containing 4% paraformaldehyde, permeabilized for 1 min in PBS containing 0.2% Triton X-100 at room temperature, and rinsed in PBS. Nuclei were stained by incubating the cells for 5 min in PBS containing 5 µg/ml bisbenzimide (Molecular Probes, Eugene, OR). The migration profile of each well was determined by counting fluorescent nuclei using Image 1 analysis software (Universal Imaging, West Chester, PA) interfaced to a microscope with an automated stage (Nikon SCU-1). Random motility coefficients were calculated by computer for both sides of the rectangular cell strip, according to Hoying and Williams (12; see also Ref. 28).

Statistical analysis. All experiments were done on at least three occasions. Independent experiments were defined as those performed on different days, with cells derived from different animals or from different passage numbers. Analysis of variance (ANOVA) was used to evaluate statistical differences between means. Bonferroni tests were used to identify between-group differences when the ANOVA was significant (P < 0.05).

RESULTS

Characterization of the antibodies and identification of subtypes present in RASM cells. Figure 1 illustrates the relative specificity and lack of cross-reactivity of the individual α₂-AR-specific antibodies. COS-7 cells were transiently transfected with the α₂A-ARs (A–C), α₂B-ARs (D–F), or α₂C-ARs (G–I). Immunofluorescent microscopy was then done using the individual antibodies. Figure 1, A, D, and G show fluorescence of cells after incubation with anti-α₂A-NH₂ polyclonal antibodies. Figure 1, B, E, and H show fluorescence of cells after incubation with anti-α₂B-2ECL polyclonal antibodies. Figure 1, C, F, and I show fluorescence of cells after incubation with anti-α₂C-NH₂ polyclonal antibodies. The antibodies appear to be specific for their individual receptor subtype and do not present any immunoreactivity to cells transfected with a different receptor subtype.

Primary cultures of RASM cells were prepared from explants and were initially characterized with respect to their morphology and the presence of smooth muscle-specific α-actin. At confluence, cell cultures displayed the typical “hill and valley” growth characteristic of vascular smooth muscle cells. Immunofluorescent labeling using a monoclonal anti-smooth muscle α-actin primary antibody showed positive labeling, as expected of these cells (data not shown). Immunofluorescence microscopy was then used to determine the expression of the α₂-ARs in RASM cells, using antibodies that were selective for either the α₂A-, α₂B-, or α₂C-AR subtypes. As shown in Fig. 2A, positive labeling of RASM cells was obtained, with antibodies directed against the α₂A-AR subtype that was blocked when the antibodies were preincubated with the fusion protein used to generate these antibodies (D). Similarly, antibodies selective for the α₂B- (B) and α₂C- (C) AR subtypes showed specific labeling that was blocked by preincubation with the corresponding fusion proteins (E and F, respectively). Thus it appears that all three α₂-AR subtypes are expressed in RASM cells. It should be noted, however, that with increasing passage number, α₂-AR immunoreactivity decreased, such that by passages 10-12 it was undetectable. For this reason, all experiments were performed with RASM cells of passages 2-6. In addition, preliminary experiments revealed that, in early passages, neither the presence or absence of serum nor the cell density affected the expression of the receptors.

To further verify the presence of the three α₂-AR subtypes in RASM cells, subtype-selective primers were designed and RT-PCR was performed using RNA isolated from these cells. The α₂-ARs are very GC rich, and GC damping is often a problem. Consequently, we used 40 PCR cycles to amplify the potential α₂-AR cDNA present. As shown in Fig. 3, RT-PCR products of the predicted size were obtained for all three subtypes. These products were absent when reactions were done with RNA that had been treated with RNase, indicating that they arose from RNA and not from contaminating genomic DNA. Linearized plasmid DNA was also incubated with RNase to verify that there was no deoxyribonuclease present.

α₂-AR-stimulated MAP kinase activity. MAP kinase activity was measured in primary cultures of RASM cells after stimulation with the α₂-AR-selective agonist Dex. Figure 4 shows the multiple stimulation of MAP kinase activity, as determined by the incorporation of 32P into myelin basic protein, after immunoprecipitation of enzyme with antibodies to both ERK 1 and ERK 2. Incubation of RASM cells with Dex resulted in a statistically significant stimulation of MAP kinase activity at a 100 nM concentration that was blocked by coincubation with the α₂B-selective antagonist RW (100 nM). The maximal stimulation, approximately fivefold at 10⁻⁶ M Dex, was comparable to the stimulation obtained with 100 nM phorbol myristate acetate. In addition, the Dex-stimulated increase in MAP kinase activity was blocked after pretreatment of the cells with a Gα₃/Go-selective inhibitor PTX.

[3H]thymidine incorporation assay. Because the stimulation of MAP kinase activity is frequently associated with cellular proliferation, we examined the effects of α₂-AR stimulation on the incorporation of [3H]thymidine. Incubation of cultured RASM cells for 24 or 48 h with 1 µM Dex did not stimulate [3H]thymidine incorporation over basal levels (Fig. 5). This was repeated under conditions (in strip) that were identical to the cell migration assays described below, and there was no stimulation of [3H]thymidine incorporation by Dex. Cells incubated with 2% FBS for 24 or 48 h did,
however, show a significant stimulation of [3H]thymidine incorporation.

$\alpha_2$-AR stimulation of RASM cell migration. Using automated video microscopy in a linear migration assay (12, 28), we calculated random motility coefficients for either untreated RASM cells, RASM cells that had been incubated for 48 h with 1 µM Dex or with 1 µM Dex plus 1 µM RW. In Fig. 6, the mean results of four independent experiments show that incubation with Dex produced an 11-fold increase in the migration of the RASM cells compared with the untreated controls. Coincubation of the RASM cells with Dex and RW blocked this increase and indicated that the effects of Dex on RASM cell migration were specific and mediated by the activation of $\alpha_2$-ARs.

Effects of $\alpha_2$-AR stimulation on F-actin polymerization. To investigate the possibility that the increase in cell motility might be associated with cytoskeletal changes, we used fluorescence microscopy to examine the extent of F-actin polymerization in RASM cells that had been incubated for 48 h with 1 µM Dex or with 1 µM Dex plus 1 µM RW. In Fig. 6, the mean results of four independent experiments show that incubation with Dex produced an 11-fold increase in the migration of the RASM cells compared with the untreated controls. Coincubation of the RASM cells with Dex and RW blocked this increase and indicated that the effects of Dex on RASM cell migration were specific and mediated by the activation of $\alpha_2$-ARs.
both high and low density. As with the effects of Dex on cell migration, the effects of Dex on F-actin polymerization were consistent with the activation of \( \alpha_2 \)-ARs.

To see if a pulse of Dex would initiate a migratory response or a change in actin labeling, experiments were performed in which cells were incubated with Dex (0.1 and 1.0 \( \mu \)M) for 5 min (as in the MAP kinase assays) and rinsed and then, 38 or 18 h later, changes in migration and actin, respectively, were monitored. However, in both of these cases, there were no differences between the pulse-treated cells and untreated cells. In addition, we looked for changes in actin immediately after a 5-min Dex pulse and saw no differences. It appears that constant Dex exposure is necessary.

**DISCUSSION**

\( \alpha_2 \)-ARs are present in cultured RASM cells, as determined by immunofluorescence microscopy, RT-PCR, and several measures of functional activation. The results of both the immunofluorescence microscopy and RT-PCR indicated that all three \( \alpha_2 \)-AR subtypes were expressed in RASM cells, although with increasing passage number, expression was lost. Functionally, the activation of RASM cell \( \alpha_2 \)-ARs stimulated MAP kinase activity in a dose-dependent and PTx-sensitive manner, increased cell migration, and decreased the intensity of F-actin labeling. These results are consistent with a possible role of \( \alpha_2 \)-ARs in vascular wound healing as well as in pathologies such as atherosclerosis and restenosis.

It was interesting to note the difference in immunofluorescent reactivity among the receptor subtypes. Although all three receptors localize diffusely along the plasma membrane, the \( \alpha_2A \)-AR appears to also exhibit a nuclear staining pattern and the \( \alpha_2C \)-AR exhibits a prominent perinuclear staining pattern. The identification of differential receptor subcellular localization is not novel and may be explained in a number of ways. For example, the receptors may exhibit different degrees of targeting, turnover, and sequestration. Daunt et al. (7) and Keefer et al. (15), with \( \alpha_2 \)-ARs, and Tarasova et al. (29) with CCK receptors have shown that G protein-coupled receptors are able to exhibit differential localization to specific plasma membrane domains (e.g., apical vs. basolateral) as well as subcellular domains. In addition, differential subcellular localization may also be a function of cell type (7).
Previous studies by Ping and Faber (23) using RT-PCR identified only the presence of the $\alpha_2A$-AR in both vascular tissue and cultured vascular smooth muscle cells from rat. Perhaps the differences in the results of the present study derive from the use of different methods of cell isolation (i.e., enzymatic dispersal vs. tissue explant) or differences in culture conditions, passage number, or primers and RT-PCR conditions. In addition, studies with transgenic mice lacking the $\alpha_2B$-AR suggest that this subtype is normally expressed in vascular smooth muscle and that its activation produces vasoconstriction (18). The knockout studies are limited, however, in that they do not look at isolated arteries or veins and cannot rule out compensation by a nontargeted subtype. The present study is the first to indicate that the $\alpha_2C$-AR is also expressed in vascular smooth muscle. Interestingly, studies with transgenic mice lacking the $\alpha_2C$ subtype showed no changes in hemodynamic parameters, which raises the question of their function in vascular tissues.

Generally, the activation of $\alpha_2$-ARs is known to have effects on a number of cellular pathways. For example,
it has been shown that the α₂-ARs mediate release of Ca²⁺ from intracellular stores (17), stimulate the secretion of prostaglandins (most likely an α₂B-AR response) (21), promote preadipocyte proliferation (3), and stimulate MAP kinase activation in stably transfected CHO cells (10). In the present studies, α₂-AR activation was also found to stimulate MAP kinase activity; however, this did not appear to be associated with any proliferative effects on the RASM cells. Besides the association of MAP kinase with proliferation, the activation of this pathway has also been associated with effects on vascular contraction, cardiac hypertrophy, and migration. To examine if there might be an effect of α₂-AR activation on cell migration, we performed an assay that determined the linear dispersion of a cell population from a defined source and provided a stochastic measure of cell migration, known as the random motility coefficient (12, 28). We found that α₂-AR activation stimulated cell migration and that this was blocked in the presence of the α₂-AR antagonist RW.

Previously, in wound-healing studies of the corneal endothelium, it has been found that the migration and spreading of cells is associated with a decrease in F-actin in the cortical region of the cells (14, 22). We examined changes in RASM cell F-actin and found that α₂-AR activation produced a dramatic decrease in TRITC-phalloidin labeled F-actin throughout the cell. These results illustrate that ARs mediate functions beyond vasoconstriction. The fact that α₂-AR stimulation had an effect on cell migration implies that they may play a role in vascular wound healing and may potentially contribute to pathologies such as atherogenesis or hypertension. In this sense, a model may be developed in which, under normal conditions, α₂A-ARs in the endothelium facilitate the release of nitric oxide to produce a net hypotensive response (in conjunction with a centrally mediated decrease in sympathetic tone), whereas the α₂-ARs in the medial smooth muscle compete by modulating vasoconstriction. Additionally, after a lesion to the intima, catecholamines from the blood would have greater access to the medial smooth muscle α₂-ARs, which could cause cellular migration and thereby contribute to the wound-healing process or, if uncontrolled, contribute to atherogenesis. An important question that still needs to be addressed is whether these effects on cell migration and cytoskeletal F-actin can be attributed to the activation of a specific α₂-AR subtype. This is particularly interesting in terms of the apparent differences in the desensitization and downregulation of the receptor subtypes. In studies by Koblika and colleagues (7, 31) and in our preliminary studies with RASM cells, it appears that there are differences between the subtypes with respect to the ability of agonists to cause receptor internalization, downregulation, and/or desensitization. Because the α₂-agonist-stimulated effects on cell migration and on F-actin typically have long time courses, it might be significant if these effects were mediated by a receptor that does not desensitize.

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