Different α-adrenoceptors mediate migration of vascular smooth muscle cells and adventitial fibroblasts in vitro

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Zhang, Hua, Carie S. Facemire, Albert J. Banes, and James E. Faber. Different α-adrenoceptors mediate migration of vascular smooth muscle cells and adventitial fibroblasts in vitro. Am J Physiol Heart Circ Physiol 282: H2364–H2370, 2002. First published January 10, 2002; 10.1152/ajpheart.00858.2001.—Norepinephrine directly induces growth of the vascular wall, which may involve not only proliferation of smooth muscle cells (SMCs) and adventitial fibroblasts (AFBs) but also augmentation of their migration. To test this hypothesis, growth-arrested SMCs and AFBs from rat aorta were exposed to norepinephrine. Norepinephrine caused dose-dependent migration of both cell types that was dependent on chemotaxis. In contrast, platelet-derived growth factor (PDGF)-BB, used as a positive control, stimulated both chemotaxis and chemokinesis. Only α1D-adrenoceptors (AR) and α2-AR antagonists inhibited norepinephrine migration of SMCs, whereas norepinephrine migration of AFBs was only inhibited by α1A-AR and α1B-AR antagonists; β-AR blockade was without effect. Norepinephrine and PDGF-BB were additive for AFB, but not SMC, migration. Stimulation of migration was reversed at high norepinephrine concentrations (10 μM); this inhibition was mediated by α2- and β-ARs in AFBs but not in SMCs. Thus norepinephrine induces migration of SMCs and AFBs via different α-ARs. This action may participate in wall remodeling and norepinephrine potentiation of injury-induced intimal lesion growth.

artery; adrenergic receptor; growth; remodeling; platelet-derived growth factor

VASCULAR WALL GROWTH and remodeling involve smooth muscle cell (SMC) and adventitial fibroblast (AFB) proliferation, hypertrophy, migration, apoptosis, and extracellular matrix changes. These mechanisms permit adaptive changes in vascular structure in response to sustained increases in arterial pressure or shear stress (reviewed in Refs. 32, 39). On the other hand, excessive wall growth and inward or inadequate outward remodeling are caused by surgical procedures, such as restenosis after angioplasty, stent placement, atherectomy, and bypass grafting. These changes in wall structure also underlie diseases such as atherosclerosis, vasculitis, systemic and pulmonary hypertension, and accelerated arteriosclerosis (32, 39). Thus the mechanisms regulating growth and migration of SMCs, AFBs, and endothelial cells are under intensive investigation.

Recent evidence suggests that catecholamines exert a direct trophic effect on vascular SMCs and AFBs. Early studies using sympathetic denervation and infusion of catecholamines and adrenergic antagonists, as well as studies correlating catecholamines with wall thickness, fibrosis, and atherosclerosis, indirectly supported this hypothesis (reviewed in Refs. 5, 16, 37, 41, 44). However, concomitant systemic hemodynamic and/or humoral effects complicated these studies. Chronic systemic α1-adrenoceptor (AR) antagonists reduced proliferation of vascular wall cells, neointimal growth, and restenosis by at least 50% in the rat and rabbit carotid after balloon injury (12, 17, 25, 38). However, whether these effects were secondary to systemic hemodynamic and humoral changes was again unclear. Recent studies suggest that these findings may derive, at least in part, from a direct trophic action of catecholamines on SMCs and AFBs. Norepinephrine (NE) stimulated rat aorta SMCs to proliferate and hypertrophy through activation of α1-ARs (5, 41, 43) and stimulated AFBs to proliferate (11). In uninjured rat aorta maintained in organ culture, NE caused medial SMCs to hypertrophy and adventitial AFBs to proliferate and reduced expression of marker proteins that characterize the differentiated SMC phenotype (44). In addition, NE strongly augmented proliferation in intima-media and adventitia by stimulation of α1A- and α1B-ARs, respectively, in aorta studied in organ culture several days after balloon injury in vivo; NE also augmented injury-induced changes in SMC marker proteins (44). Moreover, in studies employing chronic local perivascular administration to avoid systemic effects, NE augmented neointimal growth and lumen narrowing in balloon-injured rat carotid, whereas α1-AR blockade lessened these effects and adventitial thickening (10).

Migration of SMCs (31), and possibly AFBs (15, 40), to the intima plays an important role in the intimal hyperplastic complications of surgical interventions and vascular diseases such as atherosclerosis. In addition to NE’s stimulation of SMC and AFB growth,
augmentation of wall growth by NE could arise from stimulation of SMC and/or AFB migration, or from synergism of NE with migratory factors, such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) induced by vascular injury and disease (31, 32). However, although several studies suggest that NE may be capable of stimulating migration of SMCs (24, 28, 42), no studies have examined this hypothesis in SMCs and AFBs or identified the AR subtypes involved.

It has recently been shown that blood vessels express multiple AR types, some of which do not modulate vascular smooth muscle tone and whose function is thus unclear (8, 11, 37). For example, medial SMCs and adventitial AFBs of the rat thoracic aorta in vivo both express all three α1-ARs (α1A, α1B, and α1D) in the same total α1-AR abundance (11). Both cell types also express β-ARs and one of the three α2-ARs: the α2D-A (hereafter referred to as the α2-AR which is the species ortholog expressed in the rat) (11). Whereas α1D- and α2D-ARs signal constriction and β-ARs dilation of rat aorta, α1A- and α1B-ARs mediate adrenergic growth of the media and adventitia, respectively (11, 44). Therefore, the purpose of this study was to determine whether NE stimulates migration of SMCs or AFBs, and/or if it can interact with other growth and migratory factors such as PDGF-BB, and to identify the responsible AR type(s).

MATERIALS AND METHODS

Cell culture. Medial SMCs and AFBs were obtained from the descending thoracic aorta of 200-g Sprague-Dawley rats. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with high glucose containing 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 200 mg/ml l-glutamine, as described previously (11). Cells were passaged with 0.1% trypsin EDTA at ~95% confluence every 3 days (AFBs) or 5 days (SMCs) and were used in passages 3–5. When cells reached 100% confluence, they were growth-arrested by maintenance in serum-free medium (DMEM containing 0.1% BSA and 200 mg/ml l-glutamine) for 24 h before the experiment (5, 26, 41).

Migration assay. Cell migration was measured in Transwell chambers (Costar, Cambridge, MA). The top chambers with polycarbonate membranes having 8- or 12-μm pores were used for measurement of migration of AFBs or the larger SMCs, respectively. Membranes were coated with 0.1% gelatin in DMEM at 4°C overnight and dried at room temperature before use. The bottom chambers contained serum-free medium with 0.1 mM ascorbic acid to oppose oxidation of NE. Depending on the experiment, the following drugs were added to the bottom chambers: recombinant human PDGF-BB (0.5–10 ng/ml, GIBCO), NE (0.001–10 μM, Sigma), α1D-AR antagonist BMY-7378 (RB Pharma, Natick, MA), α1A-AR antagonist KMD-3213 (kindly provided by Dr. Y. Kurashina and Kissei Pharmaceutical, Matsumoto-City, Japan), α1B-AR antagonist AH-11110A (Tocris, Ballwin, MO), α2-AR antagonists atipamezole (Orion-Farmos Pharmaceutical, Turku, Finland) or RX-821002 (Tocris), and β-AR antagonist propranolol (Sigma). Final dilution of these competitive antagonists was in media, and they were present at the same 0.1 μM concentration in the top and bottom chambers. Atipamezole and RX-821002 have >1,000-fold selectivity for α2-ARs over other ARs (36, 44) and high affinity for blockade of α2D-ARs, which is the only subtype expressed in the SMCs and AFBs studied herein (11). The relative affinities and selectivities, at 0.1 μM, of the above-mentioned α1-AR antagonists, which are the most selective available, have been confirmed previously (11, 41, 44; see also Discussion). The bottom chambers were equilibrated for 30 min in the cell culture incubator before cells were placed in the top chamber. For normalization to basal (random) cell migration, all experiments included contemporary, time-matched control wells (without drugs) performed at the same n sizes as the drug treatment groups.

Serum-deprived cells were detached with 0.1% trypsin/EDTA for 1.5–2 min at 37°C, taking care to not exceed 2 min of enzyme exposure. An equal volume of soybean trypsin inhibitor (0.5 mg/ml, Worthington, Freehold, VA) was added, followed by gentle pipette trituration three times, and pelleting at 500 g for 5 min. Cells were gently washed in DMEM and resuspended in serum-free medium at a density of 2.5 × 10^6 cells/ml (AFBs) or 1.7 × 10^6 cells/ml (SMCs). Suspensions of AFBs or SMCs were added to the top chamber to give 7,500 cells/mm^2 of insert membrane, together with any test drugs. Chemotaxis (directed migration toward a positive gradient of a soluble mediator) was distinguished from chemokinesis (nondirected migration in response to a soluble mediator) by placing NE or PDGF-BB at equal concentrations in both chambers. After 6 h at 37°C in a 5% O_2/5% CO_2 incubator, the membrane was rinsed three times with PBS, and the top side was rubbed with three cotton-tipped applicators to remove adherent cells. Membranes were dried in air and fixed with methanol for 10 min at room temperature, and cells were stained with Mayer’s hematoxylin at 4°C overnight. Migrated cells present on the bottom surface were counted with an inverted microscope at ×200 magnification in four randomly selected fields per membrane.

Statistical analysis. Data are presented as means ± SE for n experiments conducted using approximately equal numbers of passage 3–5 cells for each study. Statistical significance (P < 0.05) was determined by unpaired two-tailed t-tests.

RESULTS

NE is chemotactic for SMCs and AFBs. As a positive control, PDGF-BB induced dose-dependent migration of SMCs and AFBs (Fig. 1). At the highest concentration of PDGF-BB tested (10 ng/ml), migration was completely reversed in SMCs, but not in AFBs. This inhibition of migration to PDGF-BB at high concentrations has been noted previously (6); the responsible mechanism and why it was not evident in AFBs were examined below. In contrast to PDGF-BB, NE is chemotactic for SMCs and AFBs (mechanism examined below). In contrast to PDGF-BB, migration to NE involved only stimulation of chemotaxis, and not chemokinesis, as no migration occurred when NE was in equal concentration (1 μM) on both sides of the membrane (Fig. 1).
Different α1-AR subtypes stimulate and inhibit migration of SMCs and AFBs. NE-induced migration of SMCs was inhibited by the 1D-AR antagonist BMY-7378 and the 2-AR antagonists RX-821002 or atipamezole but was unaffected by 1A-, 1B-, or 2-AR antagonists (Fig. 2). There was a trend toward propranolol inhibition of SMC but not AFB migration to NE, although this was not statistically significant despite the relatively large sample sizes for control and propranolol groups (15 and 10, respectively). In contrast, NE-induced migration of AFBs was inhibited by the 1A-AR antagonist KMD-3213 and the 1B-AR antagonist AH-11110A; however, it was unaffected by the 1D- and β-AR antagonists and was augmented by the 2-AR antagonists (Fig. 2).

The different adrenoceptors (ARs) mediate migration of SMCs (A) and AFBs (B). The bottom Transwell chamber contained 1 μM NE alone or in combination with 0.1 μM of the AR antagonists: KMD-3213 (KMD, α1A-AR antagonist), AH-11110A (AH, α1B-AR antagonist), BMY-7378 (BMY, α1D-antagonist), atipamezole or RX-821002 (ATI or RX, α2-AR antagonists, approximately equal n sizes for either antagonist), or propranolol (PROP, β-AR antagonist). Antagonists were added to both top and bottom chambers and equilibrated for 30 min before addition of NE. See legend to Fig. 1 and MATERIALS AND METHODS for additional details. Antagonists alone had no effect (see RESULTS).

 Interaction between PDGF-BB and NE for induction of AFB, but not SMC, migration. Migration of SMCs induced by an intermediate concentration of PDGF-BB (1 ng/ml) was not further increased when an intermediate migratory concentration of NE (10 nM) was also present in the bottom chamber (Fig. 3). Likewise, migration induced by a slightly higher concentration of NE (50 nM) was not further increased when given in the presence of a slightly lower concentration of PDGF-BB (0.5 ng/ml; Fig. 3). In contrast, using this same design, PDGF-BB and NE were additive for migration of AFBs (Fig. 3).

α2- and β-AR stimulation at high NE levels inhibits α1-AR-mediated migration of AFBs but not SMCs. An additional experiment was performed to test the hypothesis that reversal of AFB migration observed at high NE concentration (10 μM; Fig. 1) is due to α2-AR-mediated (and/or possibly β-AR-mediated) inhibition of the migration induced by α1A- and α1B-AR stimulation. This hypothesis was suggested by the AFB data in Fig. 2. In support of this, NE-induced inhibition of chemotaxis of AFBs at high NE concentration (10 μM) (Fig. 1) was completely reversed by the α2-AR antagonist RX-821002 and was also lessened by propranolol (Fig. 4).

In contrast, in SMCs, the inhibition of migration at high NE concentration was unaffected by RX-821002 or propranolol (Fig. 4).
DISCUSSION

These studies demonstrate that NE induces chemotactic migration of SMCs and AFBs in vitro. Different $\alpha_1$-AR subtypes mediate migration in the two cell types. Moreover, NE and PDGF-BB can act in an additive manner for migration of AFBs but not SMCs. Despite growing evidence that catecholamines have growth factor-like actions on cultured SMCs and AFBs, and on intact media and adventitia of the normal and injured vascular wall (5, 10, 11, 12, 16, 17, 25, 37, 38, 41, 43, 44), few studies have examined whether catecholamines affect migration of SMCs, and none has studied AFBs. Nishio and Watanabe (24) reported that $10^5M$ phenylephrine doubled the number of rabbit aorta SMCs present on the bottom side of a Transwell membrane when examined 24 h later and concluded that $\alpha_1$-ARs mediate SMC migration. However, it was not clear whether this represented adrenergic-induced migration or, instead, resulted from phenylephrine-induced proliferation of cells that had randomly migrated to the lower side of the membrane. Indeed, the authors had confirmed in the same study that phenylephrine induces strong SMC proliferation, as reviewed previously (5, 16, 37, 41, 43). A similar absence of differentiation between migration and proliferation makes difficult the interpretation of a more recent report that concluded that NE stimulates migration of rat aorta SMCs via $\alpha_2$-ARs (28). In a recent study (42), $10 \mu M$ phenylephrine caused a fivefold increase in migration in a modified Boyden chamber, measured over a 4-h interval, of cultured SMCs from rat renal artery. Although prazosin blockade demonstrated $\alpha_2$-AR dependence, proliferation was not arrested in these cells before seeding into migration chambers. In the present study, basal proliferation and that inducible by NE or PDGF-BB were prevented by using confluent cells that have greatly reduced proliferation rates, placing them in serum-free media for 24 h before the experiment, and limiting the duration of measurement to 6 h, which is well below the time required for proliferation of growth-arrested SMCs or AFBs after initial seeding (5, 11, 26, 41). Moreover, we have shown previously that 0.001–1 $\mu M$ NE dose dependently stimulates hypertrophy but not proliferation of quiescent SMCs in vitro (5, 41) and in vivo (11).

In agreement with our results, NE has been reported to induce migration of several nonvascular cell types. Thus the well-known stimulatory effects of sympathetic nerves on immune system function appear to involve $\alpha_{1B}$-AR-mediated migration of dendritic cells (the only $\alpha_1$-AR subtype expressed in these cells); this was demonstrated in vivo, in organ culture, and in cell culture, whereas $\alpha_2$-ARs were inhibitory (20). In addition, NE, which is released from nerves supplying lymphoid organs, induces potent in vitro migration of human monocytes and macrophages via $\beta$-ARs and cAMP (35).

![Fig. 3. NE and PDGF-BB are additive for migration of AFBs (B) but not for migration of SMCs (A). See legend to Fig. 1 and MATERIALS AND METHODS for additional details.](image)

![Fig. 4. Inhibition of migration at high NE concentration (Fig. 1) is dependent on both $\alpha_2$- and $\beta$-ARs for AFBs (B) but not for SMCs (A). The bottom Transwell chamber contained 10 $\mu M$ NE alone or in combination with 0.1 $\mu M$ of the $\alpha_2$-AR antagonist RX-221002 (RX) or the $\beta$-AR antagonist propranolol (both at 0.1 $\mu M$). Antagonists were added to both top and bottom chambers and equilibrated for 30 min before addition of NE. See legend to Fig. 1 and MATERIALS AND METHODS for additional details.](image)
The migratory efficacy of NE at AFBs and SMCs was \(~40\) and \(60\%\), respectively, of PDGF-BB. However, PDGF-BB is also more potent and efficacious than other growth factors, such as PDGF-AB, PDGF-AA, bFGF, and epidermal growth factor, that induce migration similar in maximal magnitude to NE (2). Also, growth-arrest of SMCs, as used herein, favors the differentiated phenotype (26) and is known to render SMCs (and possibly AFBs) less responsive to migratory stimuli. Thus NE migration of SMCs and AFBs appears to possess comparable efficacy to other peptide growth factors. In addition, the dose-response range for NE migration is consistent with the range over which NE regulates vascular constriction in vivo. This is consistent with the hypothesis that NE-mediated migration may occur in vivo under conditions, such as vascular injury, that render SMCs and AFBs competent for migration.

The antagonists BMY-7378, KMD-3213, and AH-11110A were used to differentiate among \(\alpha_1\)-AR subtypes. Although they do not possess the very high selectivity of RX-821002, atipamezole, and propranolol used herein for differentiating \(\alpha_2\) - and \(\beta\)-ARs, respectively, they are the most selective \(\alpha_1\)-AR subtype antagonists available (reviewed in Refs. 11, 30, 44). Reported inhibitory constant (\(K_i\)) values (in nM) for BMY-7378 at cloned rat receptors for \(\alpha_{1D}\), \(\alpha_{1B}\), and \(\alpha_{1A}\)-ARs average 1.2, 320, and 320, respectively, demonstrating \(\alpha_{1D}\)-AR selectivity of 267-fold. At cloned rat \(\alpha_{1A}\)-ARs and the submandibular gland, \(K_i\) values for KMD-3213 averaged 0.28 and showed 56- and 583-fold selectivity against \(\alpha_{1D}\) and \(\alpha_{1B}\)-ARs, respectively, and 200-fold selectivity for \(\alpha_{1A}\)-ARs over \(\alpha_{1B}\)-ARs in binding and functional studies. We confirmed the selectivity of BMY-7378 and KMD-3213 at 0.1 \(\mu\)M for blockade of \(\alpha_{1D}\)- and \(\alpha_{1A}\)-ARs in radioligand binding and functional (growth) studies of rat aorta SMCs and AFBs and three Rat1 fibroblast cell lines each transfected with one of the \(\alpha_1\)-AR subtypes (11, 41, 44).

In contrast to the appreciable selectivity of BMY-7378 and KMD-3213, the \(K_i\) for AH-11110A at the cloned \(\alpha_{1B}\)-AR is 79.4 nM, with 32- and 26-fold selectivity over \(\alpha_{1A}\) - and \(\alpha_{1D}\)-ARs, respectively; this agrees with a similar 10- to 20-fold selectivity reported in a functional study (\(\alpha_{1B} > \alpha_{1A} > \alpha_{1D}\)) (9, 14, 30). However, despite this only moderate selectivity, we previously found in cells identical in passage number and serum withdrawal treatment as used herein that total \(\alpha_1\)-AR density in SMCs is twice that in AFBs and that in SMCs \(\alpha_{1B}\)-ARs are twofold more abundant than \(\alpha_{1D}\)-ARs, whereas in AFBs, \(\alpha_{1B}\)-ARs are fourfold more abundant than \(\alpha_{1D}\)-ARs (11). In both cell types, \(\alpha_{1A}\)-ARs were below detection by competition binding assays, but mRNA levels were almost identical. While the approximately threefold greater density of \(\alpha_{1D}\)-ARs on SMCs than on AFBs could account for the reliance on \(\alpha_{1D}\)-ARs for migration of SMCs, the absence of any detectable effect of BMY-7378 on AFBs is not consistent with the hypothesis that differences in receptor density underlie the results obtained herein. Moreover, \(\alpha_{1B}\)-ARs are more abundant than \(\alpha_{1D}\)-ARs in both cell types. In addition, SMCs and AFBs express similar levels of \(\alpha_{1A}\)-AR mRNA, yet KMD-3213 abolished SMC migration but had no effect in SMCs. It is possible that the small density of \(\alpha_{1A}\)-ARs on AFBs are either well coupled to migratory signaling pathways or are upregulated by exposure to NE to explain our results; however, these mechanisms would also have to be nonexistent in the SMCs to be consistent with our findings. Such agonist-induced upregulation of the \(\alpha_{1A}\)-AR, but not \(\alpha_{1B}\)- or \(\alpha_{1D}\)-AR, has been reported in neonatal rat cardiomyocytes (29) and not in the SK-N-MC cell line (33). Differences in receptor abundance among SMCs and AFBs also do not appear to underlie why \(\alpha_{2D}\)-ARs promote migration in SMCs but inhibit migration of AFBs, and why \(\beta\)-ARs inhibit migration of AFBs, at least at high NE levels (>1 \(\mu\)M; it is possible that lower levels of NE stimulated \(\beta\) - and \(\alpha_{2D}\)-ARs and lessen the \(\alpha_{1A}\)- (and possibly \(\alpha_{1B}\)-AR-induced AFB migration). Levels of \(\alpha_{2D}\)-AR expression (the only \(\alpha_2\)-AR subtype detected) are similar in cultured rat...
aorta SMCs and AFBs (11). Likewise, β-AR density is higher in media than adventitia (reviewed in Ref. 11), yet β-AR stimulation inhibited migration of AFBs but not SMCs. Thus differences in α- and β-AR abundance between SMCs and AFBs do not correlate with the differences in the AR types that we have found promote and oppose their migration.

With the assumption that the fundamental capacity to migrate, which is exhibited by most cells, relies on similar intracellular signaling pathways in SMCs and AFBs, a possible explanation for the cell-specific AR types influencing their migration is that the divergent postreceptor effector pathways activated by different ARs (13, 27, 37, 42) may differ between the two cell types in their interaction with pathways signaling migration. It is also possible that signaling pathways for the same AR type, as well as those mediating migration itself (31, 34), may differ for SMCs and AFBs. However, there is no information on these pathways in AFBs for comparison with those described for SMCs. In addition to the different AR types modulating migration in SMCs and AFBs, migration to PDGF-BB and NE were additive in AFBs but not in SMCs. The surprising absence in SMCs suggests that postreceptor pathways for NE and PDGF-BB migration in SMCs are not parallel but are such that stimulation of one pathway blocks simultaneous signaling through the other pathway. Clearly, future studies are needed to determine if the present findings occur in vivo, how adrenoceptor and migration effector pathways interact in SMCs and AFBs, and whether adrenergic-induced migration is important in vascular remodeling and disease.

We have previously demonstrated that catecholamines are directly trophic, i.e., NE stimulates proliferation and hypertrophy in cultured SMCs and AFBs and in the intact wall in organ culture and in vivo and promotes the dedifferentiated phenotype in vivo; moreover, these effects are strongly augmented after vascular injury (5, 11, 41, 44). In addition, local blockade of α1-ARs attenuates, and local increase in NE levels augments, neointimal expansion and restenosis after balloon injury (10). The present results suggest the hypothesis that worsening of neointimal expansion by catecholamines may also be dependent on NE-induced chemotaxis of SMCs and AFBs, particularly in the injured vascular wall where SMCs and AFBs are known to be competent to respond to migratory factors, and where PDGF-BB is increased, along with many other mediators, and is central in promoting migration (31, 32). However, this hypothesis requires consideration of NE gradients across the vascular wall. In uninjured vessels, NE release from adrenergic nerves, that are concentrated at the external elastic lamina, diffuses toward the much lower concentration of NE in plasma [≈1 nM at rest, ~10-fold increase with strong physiological stresses (18, 19)]. Several observations suggest that this gradient may be reversed by certain types of injury. Vascular nerves become undetectable by immunohistochemistry in the rat carotid 1 day after balloon injury but are fully restored when examined 28 days later (22). Denervation also occurs after organ transplantation or vascular bypass grafting. In addition, various types of wall injury induce accumulation of activated platelets and monocyte/macrophages (and lesser numbers of neutrophils and T lymphocytes) at the intima and inner media (31, 32). All of these cells are able to take up and/or synthesize NE (1, 7, 21, 23), which may underlie the threefold increase in wall NE content when measured 28 days after balloon injury (3). Thus mechanical injury (and possibly other types of injury) may transiently reverse the gradient, allowing NE to contribute to migration of SMCs and AFBs that underlies intimal expansion (10, 11, 32, 43). Therefore, not only NE-mediated proliferation of SMCs and AFBs, dedifferentiation of SMCs, and accumulation of collagen (10, 44), but also migration, could explain how catecholamines worsen restenosis in animal models (10, 12, 17, 25, 38, 44).

In conclusion, NE stimulated chemotactic migration of SMCs and AFBs that appears to be mediated by α1D- and α2D-ARs in SMCs and α1A-ARs (and possibly α1B-ARs) in AFBs. Simulation of α2D- and β-ARs opposed migration of AFBs but not SMCs. Thus alterations in expression of AR types may have significant effects on modulation of migration by catecholamines. Whether noradrenergic migration of SMCs and AFBs occurs in vivo, depends on the same ARs, and contributes to wall remodeling in physiological adaptation, vascular diseases, or surgical complications will require development of in vivo methods to trace cell migration and to selectively activate and inhibit the multiple AR subtypes expressed. Affirmative findings would raise the possibility that migration of SMCs and/or AFBs may be suppressed for therapeutic advantage using AR subtype-selective antagonists.

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REFERENCES


