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Inhibitory effect of D₁-like and D₃ dopamine receptors on norepinephrine-induced proliferation in vascular smooth muscle cells

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Abstract

The sympathetic nervous system plays an important role in the regulation of blood pressure. There is increasing evidence for positive and negative interactions between dopamine and adrenergic receptors; the activation of the α -adrenergic receptor induces vasoconstriction, whereas the activation of dopamine receptor induces vasorelaxation. We hypothesize that the D₁-like receptor and/or D₃ receptor also inhibit α ₁-adrenergic receptor-mediated proliferation in vascular smooth muscle cells (VSMCs). In this study, VSMC proliferation was determined by measuring [³H]thymidine incorporation, cell number, and uptake of 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT). Norepinephrine increased VSMC number and MTT uptake, as well as [³H]thymidine incorporation via the α ₁-adrenergic receptor in aortic VSMCs from Sprague-Dawley rats. The proliferative effects of norepinephrine were attenuated by the activation of D₁-like receptors or D₃ receptors, although a D₁-like receptor agonist, fenoldopam, and a D₃ receptor agonist, PD-128907, by themselves, at low concentrations, had no effect on VSMC proliferation. Simultaneous stimulation of both D₁-like and D₃ receptors had an additive inhibitory effect. The inhibitory effect of D₃ receptor was via protein kinase A, whereas the D₁-like receptor effect was via protein kinase C- ζ . The interaction between α ₁-adrenergic and dopamine receptors, especially D₁-like and D₃ receptors in VSMCs, could be involved in the pathogenesis of hypertension.

Keywords

α ₁-adrenergic receptor; hypertension

Cardiovascular disease remains the major cause of death in the world, and hypertension accounts for the major cause of these deaths in the United States and Western Europe (46). Hypertension is known to be associated with an increase in sympathetic activity (8,42). The

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sympathetic neurotransmitters, norepinephrine and epinephrine, play important roles in the regulation of physiological and pathological processes in the cardiovascular system, via binding to adrenoceptors (21). Vascular smooth muscle cell (VSMC) proliferation is central to the development of vascular diseases, such as restenosis and atherosclerosis. In addition to the fact that prolonged elevation of plasma catecholamines is a risk factor for vascular diseases, recent reports have shown that catecholamines directly induce hypertrophy of the arterial wall by stimulation of α_1 -adrenoceptors (7,10). Catecholamines in cell and organ culture induce dose-dependent proliferation of VSMCs, and the trophic effect is dependent on the production of reactive oxygen species (5). Furthermore, the potency of these effects is strongly augmented in hypertensive states (47,55,60).

Although dopamine is a precursor of norepinephrine and epinephrine, by itself it also exerts widespread effects both in neuronal and nonneuronal tissues (18,57). Dopamine receptors, classically, have been divided into D₁- and D₂-like subtypes based on their interaction with the effector enzyme, adenylyl cyclase. The D₁-like receptors, which stimulate adenylyl cyclases, are composed of the receptor subtypes D₁ and D₅ receptors, whereas the D₂-like receptors, which inhibit adenylyl cyclase, are composed of the subtypes D₂, D₃, and D₄ receptors (18,57). Both D₁-like and D₃ receptors have been found in conduit and resistance arteries, including the aorta, mesenteric artery, and renal artery (2,34,36,53,54,60).

Dopamine, at low concentrations, via D₁-like receptors, relaxes blood vessels and decreases blood pressure (18,57). We have reported that in the rat mesenteric artery, D₃ receptor agonist has no effect on vascular contractility. However, when the mesenteric rings are precontracted with potassium chloride or norepinephrine, stimulation of D₁-like receptors or the D₃ receptor causes vasorelaxation (60). When compared with vessels precontracted with norepinephrine, the D₁-like receptor- and D₃ receptor-mediated vasorelaxation is greater in vessels precontracted with norepinephrine than in vessels precontracted with potassium chloride, indicating that norepinephrine could impair the vasodilatory effect of dopamine (60).

Dopamine also has antiproliferative effects, via D₁-like receptors in neuronal and VSMCs (41,53,54). Dopamine receptors have been reported to inhibit or stimulate cell proliferation that is subtype dependent (3,27). We hypothesize that in addition to opposing the vasoconstrictor effect of norepinephrine in the artery, activation of D₁-like and D₃ receptors reduces the proliferative effects mediated by norepinephrine, via α_1 -adrenergic receptor. To test this hypothesis, we used VSMCs from the aorta of Sprague-Dawley (SD) rats. We found that the activation of D₁-like or D₃ receptor reduces the proliferative effects mediated by norepinephrine, via the α_1 -adrenergic receptor; costimulation of D₁-like and D₃ receptors has an additive antiproliferative effect in VSMCs from SD rats. Moreover, we found that protein kinase A (PKA) is involved in the D₃ receptor-mediated antiproliferative effect, whereas protein kinase C (PKC) is involved in the D₁-like receptor-mediated antiproliferative effect.

Methods

Aortic smooth muscle cell isolation and culture

The tunica media were dissected from freshly harvested aortic strips from 200-g SD rats and plated in 100-mm petri dishes using standard explant techniques (26). Briefly, the aorta was incised longitudinally, and the endothelial cells were removed by vigorously scraping the surface of the lumen with a scalpel blade to expose the medial layer of the vessel. The vessel was then divided into 1-cm sections and placed lumen-side down in 60-mm petri dishes containing VSMC medium [Dulbecco's modified Eagle's medium (DMEM; GIBCO) supplemented with 20% FBS (HyClone) and 1% penicillin/streptomycin/bFGF/insulin/EGF (all from Sigma, St. Louis, MO)] and cultured at 37°C in a humidified 5% CO₂-95% room air atmosphere for 2 wk to allow for VSMC migration from the vessel onto the dish. Explant-

derived cells were initially treated with 0.1% trypsin, and 4 mM EDTA for 3 min at 37°C, and thereafter the passage was performed conventionally. Cells from *passages 5–15* were used for all growth studies. The cells were characterized as smooth muscle cells by morphology (multilayer sheets, “hills and valleys”) and immunostaining with monoclonal antibody specific for smooth muscle α -actin. Cells were subcultured into 24-well plates in a medium containing 10% serum for 24 h with an initial density of 5×10^4 cells per well. The cells were then placed in the medium without serum for 24 h to render them quiescent. All experiments were approved by the Third Military Medical University Animal Use and Care Committee.

Evaluation of cell number

VSMCs were seeded in six-well plastic culture dishes at a density of 1×10^4 /well in DMEM containing 10% FBS and cultured for 24 h. After the induction of quiescence, the medium was replaced with serum-free medium with the indicated drug concentrations (fenoldopam, 10^{-7} M; PD-128907, 10^{-7} M; and norepinephrine, 10^{-6} M) and incubated for another 24 h. The experiment was terminated by aspiration of the medium and washing with phosphate-buffered saline (PBS) followed by the addition of 1 ml of 2% (wt/vol) trypsin to each dish. After the collection of the cells, viable cells, determined by the uptake of 0.4% trypan blue after 5 min of mixing (Invitrogen Life Technologies), were counted using a hemocytometer (trypan blue uptake was observed in <10% of the cells). Counting was performed in triplicate (35,49).

MTT assay

The number of viable cells in each well was also estimated by the uptake of the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT). MTT, selectively taken up by mitochondria, is converted to a dark-blue product by living but not by dead cells. After the induction of quiescence in 96-well plastic culture dishes at the density of 1×10^3 cells/well, the cells were incubated with the indicated drugs for 24 h. Subsequently, 20 μ l of MTT (2.5 g/l) were added to each well, and the incubation continued for an additional 4 h at 37°C. Thereafter, 150 μ l DMSO were added to each well, and an absorbance at 490 nm was read on a Microplate reader (model 680, Bio-Rad) (25,26).

[³H]thymidine incorporation

Cell proliferation was determined by measuring the incorporation of [³H]thymidine (Atomic Energy Research Establishment of China, Beijing City, China) into DNA of cells cultured in 96-well plates. After the induction of quiescence, the cells were stimulated with norepinephrine (Huofen, Shanghai City, China), vehicle (distilled H₂O) in the presence or absence of the indicated drugs; PD-128907 (Sigma), a D₃ receptor agonist; and fenoldopam (Sigma), a D₁-like receptor agonist, for 24 h. All of antagonists or inhibitors were added into the medium 30 min before the corresponding agonists or activators. [³H]thymidine (1 μ Ci/ml) was then added to the growth medium of each well 6 h before the measurements. At the end of incubation, the medium was removed and the cells were treated with 0.25 ml of 0.05% trypsin-0.53 mM EDTA (GIBCO, Grand Island, NY) for 5 min and diluted to 10 ml with a balanced electrolyte solution (17,26). The cells were then treated with 10% trichloroacetic acid to precipitate acid-insoluble materials from which the DNA was extracted with 0.1 M NaOH. The DNA was collected on a Whatman GF/B filter and washed twice with 5 ml ice-cold PBS. The filter was then cut and shaken in 3.5 ml scintillation fluid for 24 h before counting in a liquid scintillation counter (Beckman LS6500, Beckman). Data are presented as [³H]thymidine uptake per microgram of protein.

To determine the specificity of the D₁-like and D₃ receptor agonists, D₁-like receptor and D₃ receptor antagonists, SCH-23390 (Sigma) and U-99194A (Research Biochemicals International, Natick, MA), respectively, were used to block the effect of fenoldopam or PD-128907. There are no ligands, agonists, or antagonists that can distinguish between D₁ and

D₅ receptors; therefore, the receptors involved in this experiment are D₁-like receptors (D₁ and D₅), which are expressed in the VSMCs of rats. To determine whether the proliferative effect of norepinephrine is exerted at the α_1 -adrenergic receptor, the α_1 -adrenergic receptor antagonist prazosin (Sigma) was used.

Determination of the second messenger(s) involved in the antiproliferative effects of D₁-like and D₃ receptors

To determine the second messenger(s) involved in the effect of D₁-like and D₃ receptors, cell permeable, myristoylated peptide inhibitors of PKC (peptide 19–31) (20), PKA (14–22 amide) (6) (Calbiochem, Darmstadt, Germany), and myristoylated PKC- ζ pseudosubstrate inhibitor (Calbiochem, Darmstadt, Germany) (37,51) were used. To determine the specificity of PKC and PKA inhibitors, PKC activator, phorbol 12-myristate 13-acetate (PMA, Sigma), and PKA activator, 8-(4-chlorophenylthio) adenosine-3',5'-cyclic monophosphorothioate, Sp-isomer sodium salt (Sp-cAMP[S], Sigma) were used.

Statistical analysis

The data are expressed as means $\pm$ SE. The comparison within groups was made by repeated-measures ANOVA (or paired *t*-test when only 2 groups were compared), and the comparison among groups (or *t*-test when only 2 groups were compared) was made by factorial ANOVA and Duncan's test. A value of $P < 0.05$ was considered significant.

Results

Norepinephrine, via the α_1 -adrenergic receptor, increases proliferation of VSMCs from rat aorta of SD rats

Treatment of VSMCs with varying concentrations of norepinephrine (10^{-9} – 10^{-6} M) for 24 h resulted in a concentration-dependent increase in [³H]thymidine incorporation in aortic VSMCs from SD rats (data not shown). The increase in [³H]thymidine incorporation, induced by norepinephrine, was blocked by the α_1 -adrenergic receptor antagonist, prazosin (Fig. 1), indicating that the norepinephrine-mediated proliferation was via the α_1 -adrenergic receptor.

The norepinephrine-mediated proliferation of VSMCs from rat aorta of SD rats is attenuated by a D₁-like receptor agonist

To investigate whether there is an interaction between α_1 -adrenergic and D₁-like receptors, VSMCs were incubated with norepinephrine and the D₁-like receptor agonist, fenoldopam, for 24 h. Fenoldopam, by itself, had no effect on [³H]thymidine incorporation (data not shown), but dose-dependently (10^{-12} – 10^{-5} M) reduced the stimulatory effect of norepinephrine (Fig. 2A). The effect of fenoldopam was via the D₁-like receptor, because the inhibitory effect of fenoldopam on norepinephrine-mediated proliferation was blocked by the D₁-like receptor antagonist, SCH-23390 (Fig. 2B).

The PKC inhibitor peptide 19–31 (10^{-6} M), by itself, had no effect on VSMC proliferation. The PKC inhibitor also did not affect norepinephrine-mediated VSMC proliferation but blocked the inhibitory effect of fenoldopam on norepinephrine-mediated proliferation (Fig. 2C), indicating that PKC was involved in the inhibitory action of fenoldopam (20). We also used a PKA inhibitor (14–22 amide) and a calcium channel blocker (nicardipine) in this experiment. However, neither 14–22 amide nor nicardipine could block the effect of D₁-like receptor on VSMC proliferation (data not shown).

To further investigate which isoform of PKC was involved in the mechanism, myristoylated PKC- ζ pseudosubstrate inhibitor (10^{-5} M) was used in this experiment. It resulted that myr-RRGARRWRK, by itself, had no effect on VSMC proliferation but blocked the inhibitory

effect of fenoldopam on norepinephrine-mediated proliferation (Fig. 2D), indicating that PKC- ζ was involved in the inhibitory action of fenoldopam.

The specificity of PKC inhibitor, peptide 19–31, on norepinephrine-mediated proliferation was also determined. The PKC activator, PMA, by itself, had no effect on VSMC proliferation but reduced the norepinephrine-mediated proliferation in aortic VSMCs from SD rats, which was blocked by the PKC inhibitor peptide 19–31 (Fig. 2E).

The norepinephrine-mediated proliferation of VSMCs from rat aorta of SD rats is reduced by a D₃ receptor agonist

To determine whether there is any effect of D₃ receptors on cell proliferation, the VSMCs from SD rats were treated with varying concentrations (10^{-8} – 10^{-5} M) of the D₃ receptor agonist, PD-128907 (58). We found that at low concentrations (10^{-8} and 10^{-7} M), PD-128907 had no effect on cell proliferation but at high concentrations (10^{-6} and 10^{-5} M), PD-128907 stimulated VSMC proliferation (data not shown). The stimulatory effect of PD-128907 on VSMC proliferation was blocked by the α_1 -adrenergic receptor blocker prazosin, but not by D₃ receptor antagonist U-99194A, indicating that the stimulatory effect of the high concentrations of PD-128907 is via the α_1 -adrenergic receptor rather than the D₃ receptor (control = $2,399 \pm 180$; 10^{-5} M PD-128907 = $3,824 \pm 394$; 10^{-5} M PD-128907 + 10^{-6} M U-99194A = $4,062 \pm 349$; 10^{-5} M PD-128907 + 10^{-6} M prazosin = $2,449 \pm 172$; 10^{-6} M U-99194A = $2,391 \pm 181$; 10^{-6} M prazosin = $2,424 \pm 171$ counts·min⁻¹·mg protein⁻¹, $n = 5$ experiments/group).

To investigate whether there is an interaction between α_1 -adrenergic and D₃ receptors, VSMCs were incubated for 24 h with norepinephrine (10^{-6} M) and low concentrations of the D₃ receptor agonist PD-128907. PD-128907 (10^{-12} – 10^{-7} M), by itself, had no effect but reduced the stimulatory of norepinephrine on [³H]thymidine incorporation in aortic VSMCs from SD rats (Fig. 3A). The effect of PD-128907 was via the D₃ receptor, because the inhibitory effect of PD-128907 on norepinephrine-mediated proliferation was blocked by D₃ receptor antagonist U-99194A (Fig. 3B).

The PKA inhibitor 14–22 (10^{-6} M), by itself, had no effect on VSMC proliferation. The PKA inhibitor also did not affect norepinephrine-mediated VSMC proliferation but blocked the inhibitory effect of PD-128907, indicating that PKA was engaged in the inhibitory action of PD-128907 (Fig. 3C). We also used a PKC inhibitor (19–31) and a calcium channel blocker (nicardipine) in this experiment. However, neither 19–31 nor nicardipine could block the effect of D₃ receptor on VSMC proliferation (data not shown).

The specificity of PKA inhibitor 14–22 on norepinephrine-mediated proliferation was also determined in this experiment. PKA activator, Sp-cAMP[S], by itself had no effect on VSMC proliferation but reduced the norepinephrine-mediated proliferation in aortic VSMCs from SD rats, which was blocked by the PKA inhibitor 14–22 (Fig. 3D).

D₁-like and D₃ receptors have additive inhibitory effect on norepinephrine-mediated VSMC proliferation

In additional studies, we found that costimulation with D₁-like and D₃ receptor agonists, fenoldopam (10^{-7} M) and PD-128907 (10^{-7} M), reduced norepinephrine-mediated proliferation to a greater degree than those observed with fenoldopam or PD-128907, indicating an additive effect of D₁-like and D₃ receptors (Fig. 4A).

To substantiate the above-mentioned phenomena determined by [³H]thymidine incorporation, we investigated VSMC proliferation further by counting the number of viable cells, determined by counting 0.4% trypan blue-positive cells and by the MTT method. In agreement with the [³H]thymidine incorporation method, norepinephrine increased the number of VSMCs that

was reduced in the presence of D₁-like and D₃ receptor agonists, fenoldopam (10^{-7} M) and PD-128907 (10^{-7} M), respectively. Moreover, costimulation with fenoldopam and PD-128907 reduced the norepinephrine-mediated increase in cell number to a greater extent than those observed with fenoldopam or PD-128907, indicating an additive effect of D₁-like and D₃ receptors (Figs. 4, B and C).

Discussion

There are several novel observations in our study. We show that the proliferative effect of norepinephrine, via an α_1 -adrenergic receptor, in VSMCs from the aorta of SD rats is reduced by the activation of D₁-like or D₃ receptors, although the D₁-like and D₃ receptors, by themselves, have no effect on VSMC proliferation. The D₁-like and D₃ receptors have an additive inhibitory effect on norepinephrine-mediated proliferation of VSMCs. The inhibitory effect of D₃ receptor is via PKA, whereas D₁-like receptor effect is via PKC.

The sympathetic neurotransmitters, norepinephrine and epinephrine, via α -adrenergic receptors, induce vasoconstriction and proliferation of VSMCs, whereas the opposite effects (vasodilation and inhibition of the proliferation of VSMCs) occur via β -adrenergic receptors (16,60). Dopamine at low concentrations, via dopamine receptors, dilates the resistance and capacitance arteries (18,23,57); at high concentrations, dopamine stimulates other G protein-coupled receptors, including α,β -adrenergic receptors (18,57) and serotonin receptors (14, 50).

There are increasing pieces of evidence for interactions among catecholamines and their receptors in neural and non-neural tissues. The D₂-like receptors, D₂, D₃, and D₄, are located pre- and postsynaptically/junctionally (2,30,45,60). Prejunctional D₂ dopamine receptors in postganglionic sympathetic nerves innervating various organs, including human gastric and uterine arteries, can inhibit the release of catecholamine (28). In contrast, the blockade of the presynaptic α_2 -adrenergic receptor enhances brain cortical dopamine output (15). An activation of human peripheral blood mononuclear cells triggers endogenous production of catecholamines, an effect that is blocked by an activation of D₁-like receptor. The counterregulatory actions of the α -adrenergic and dopamine receptors extend to the effects on vascular proliferation.

Our previous study showed that D₁ and D₃ receptors exist in the artery. Stimulation of D₁-like or D₃ receptor can increase both receptor protein expression in rat aorta A10 cells; the activation of D₁-like or D₃ receptor relaxes rat mesenteric arteries precontracted by potassium chloride, and the simultaneous stimulation of D₁-like and D₃ receptors has an additive relaxant effect (60). The present results show that the D₁-like or D₃ receptor has an inhibitory effect on norepinephrine-induced VSMC proliferation, and costimulation of D₁-like and D₃ receptors has an additive inhibitory effect, which further indicates the interaction between D₁-like and D₃ receptors in the artery.

α_1 -Adrenergic receptors, like dopamine receptors, are members of the class A of G protein-coupled receptors and mediate many of the physiological effects of catecholamines. The α_1 -adrenergic receptor plays a particularly important role in the regulation of blood pressure via the activation of smooth muscle replication and contraction. Consistent with our findings in this study, the activation of α_1 -receptors stimulates vascular smooth muscle growth (19). Human VSMCs express at least three subtypes of α_1 -receptors, namely, α_{1A} , α_{1B} , and α_{1D} -receptors. It is generally accepted that the activation of all three subtypes of α_1 -receptors increases the hydrolysis of phosphatidylinositol 4,5-bisphosphate to form inositol 1,4,5-trisphosphate and diacylglycerol via G_q, leading to the activation of PKC and an increase in intracellular Ca²⁺ (29). Substantial evidence indicates that the activation of PKC or Ca²⁺

channel is involved in the α_1 -adrenergic receptor induction of mitogenesis in VSMCs (22). In this study, we show that the activation of D₁-like or D₃ receptors has an inhibitory effect on norepinephrine-induced proliferation in VSMCs. However, the mechanism(s) of the antiproliferative action of D₁-like or D₃ receptor is not clear. Our studies show that the inhibitory effect of D₃ receptor is blocked by treatment with PKA inhibitor, whereas the D₁-like receptor inhibitory effect is blocked by the inhibition of PKC activity, indicating that PKA and PKC are involved in these actions. High concentrations of D₁-like or D₃ receptor agonists can also compete with norepinephrine for adrenergic receptor occupancy to some degree. However, our studies used low concentrations of D₁-like or D₃ receptor agonists that can be blocked by their respective antagonists but not by an α_1 -adrenergic receptor antagonist. However, it is interesting to find that PKA and PKC are involved in the D₁-like and D₃ receptor-mediated antiproliferative effects.

PKC isoforms are classified into four groups, the conventional PKCs (α , β I, β II, and γ), the novel PKCs (δ , ϵ , η , and θ), the atypical PKCs (ζ and λ /i), and PKD or PKC- μ . In this study, we could not determine which one is involved in the antiproliferative effects by D₁-like receptor. The activities of novel and atypical PKCs are not affected by Ca^{2+} , whereas conventional PKCs can bind Ca^{2+} (39,44). In renal proximal tubule cells, the activation of D₁-like receptor can stimulate (θ and ζ) or inhibit (δ) new PKCs or translocate specific PKC isoforms from cytosol to membrane (α , β , and ϵ) and membrane to cytosol (δ) (9,32,52,56). There are reports that PKC- β I, PKC- δ , PKC- ϵ , PKC- ζ , and PKC- η have proliferative VSMC effects (1,56). In our studies, a Ca^{2+} channel blocker has no effect on the α_1 -adrenoceptor-mediated proliferation effect; therefore, it is possible that atypical and novel PKCs, and not conventional PKCs, are involved in this action. In this experiment, we found that the inhibition of PKC- ζ blocks the inhibitory effect of fenoldopam on norepinephrine-mediated proliferation, indicating that PKC- ζ is involved in the inhibitory action of fenoldopam.

Our present study found that the D₃ receptor mediated the inhibition of VSMC proliferation is via PKA. Whereas the D₃ receptor is usually linked to adenylyl cyclase inhibition, the D₃ receptor can also be linked to its stimulation (33). The D₁-like receptor-mediated inhibitory effect on VSMC proliferation is blocked by the inhibition of PKC activity, indicating that PKC is involved in this action. This is in contrast to the study of Yasunari et al. (53), who found that the antiproliferative effect of D₁-like receptors is mediated, in part, by the inhibition of PKC activity. The reason for this difference between these two studies is not known; however, there are differences between our study and those reported by Yasunari et al. (53). First, we used VSMCs from the aorta of SD rats, whereas Yasunari et al. used VSMCs from the renal artery of Wistar rats. The differences may be vessel specific (conduit and resistance vessels or differences among resistance vessels, e.g., mesenteric vs. renal VSMCs). It should be noted, however, that it would be unlikely for D₁ receptors to inhibit PKC activity because the D₁ receptor, by itself, or via an interaction with the D₂ receptor (24,40) has been consistently reported to stimulate and not inhibit phospholipase C, the enzyme proximal to PKC activation, initially reported by Felder et al. in renal tubules (4,11,12) and subsequently confirmed in other tissues, including neural tissue (31,48) and VSMCs (43). D₁-like receptors have also been reported to stimulate (e.g., PKC- ϵ) (10,14,33) or inhibit (e.g., PKC- δ) specific PKC isoforms (52,59) that are cell specific. Second, the proliferative factors are different: we used norepinephrine, whereas Yasunari et al. used PDGF.

In summary, we have demonstrated that the proliferative effect of norepinephrine, via the α_1 -receptor, on VSMCs from rat aorta is reduced by the activation of D₁-like or D₃ receptors. A simultaneous stimulation of D₁-like and D₃ receptors has an additive inhibitory effect on norepinephrine-mediated proliferation of VSMCs. The inhibitory effect of D₃ receptor is via PKA, whereas the D₁-like receptor effect is via PKC- ζ .

Perspectives

VSMC proliferation plays an important role in the pathogenesis of hypertension. Norepinephrine, via the α_1 -receptor, increases VSMC proliferation, whereas dopamine, via the D_1 -like and D_3 receptors, reduces norepinephrine-mediated proliferative effects. The development of drugs, which selectively activate dopamine receptors, might be helpful to prevent vessel wall hypertrophy and hyperplasia, reduce peripheral arterial resistance, and normalize blood pressure.

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Fig. 1.

Effect of the α_1 -adrenergic receptor antagonist prazosin on norepinephrine (NE)-mediated proliferation of aortic vascular smooth muscle cells (VSMCs) from Sprague-Dawley (SD) rats. VSMC proliferation was determined by [^3H]thymidine incorporation after incubation with the indicated concentrations of NE (10^{-6} M) with or without the presence of α_1 -adrenergic receptor antagonist prazosin (10^{-6} M). Results are expressed as counts per minute (cpm) per mg protein ($n = 5$ experiments, $*P < 0.05$ vs. others; ANOVA, Duncan's test).

Fig. 2.

Effect of dopamine D_1 -like receptor on NE-mediated proliferation of aortic VSMCs from SD rats. **A:** effect of D_1 -like receptor agonist fenoldopam (Fen) on NE-mediated proliferation of aortic VSMCs from SD rats. VSMC proliferation was determined by ^3H -thymidine incorporation after incubation with the indicated concentrations of NE (10^{-6} M) with or without the presence of Fen (10^{-12} – 10^{-5} M). **B:** effect of a D_1 -like receptor agonist Fen and D_1 -like receptor antagonist SCH-23390 (SCH) on NE-mediated proliferation of aortic VSMCs from SD rats. The cells were incubated with the indicated reagents: Fen (10^{-7} M) and SCH (10^{-7} M) for 24 h. VSMC proliferation was determined by ^3H -thymidine incorporation. **C:** effect of PKC inhibitor, peptide 19–31 (PKCI), on the inhibitory effect of D_1 -like receptor in aortic

VSMCs from SD rats. The cells were incubated with the indicated reagents: NE (10^{-6} M), Fen (10^{-7} M), and PKCI (10^{-6} M) for 24 h. VSMC proliferation was determined by [3 H]thymidine incorporation. *D*: effect of PKC- ζ inhibitor (PKCzI) on the inhibitory effect of D₁-like receptor in aortic VSMCs from SD rats. The cells were incubated with the indicated reagents: NE (10^{-6} M), Fen (10^{-7} M), and PKCzI (10^{-5} M) for 24 h. VSMC proliferation was determined by [3 H]thymidine incorporation. *E*: effect of PKC activator, PMA and PKC inhibitor, PKCI on NE-mediated proliferation of aortic VSMCs from SD rats. The cells were incubated with the indicated reagents: NE (10^{-6} M), PKCI (10^{-6} M), and PMA (10^{-7} M) for 24 h. VSMC proliferation was determined by [3 H]thymidine incorporation. All results are expressed as cpm/mg protein [$n = 10$ (A), $n = 5$ (B), $n = 10$ (C), $n = 6$ (D), and $n = 6$ (E) experiments]. * $P < 0.05$ vs. control; # $P < 0.05$ vs. NE (ANOVA, Duncan's test).

Fig. 3.

Effect of D₃ receptor on NE-mediated proliferation of aortic VSMCs from SD rats. **A:** effect of D₃ receptor agonist PD-128907 (PD) on NE-mediated proliferation of aortic VSMCs from SD rats. VSMC proliferation was determined by [³H]thymidine incorporation after incubation with the indicated concentrations of NE (10⁻⁶ M) with or without the presence of PD (10⁻¹²-10⁻⁷ M). **B:** effect of a D₃ receptor agonist PD and D₃ receptor antagonist U-99194A (U) on NE-mediated proliferation of aortic VSMCs from SD rats. The cells were incubated with the indicated reagents: PD (10⁻⁷ M) and U (10⁻⁷ M) for 24 h. VSMC proliferation was determined by [³H]thymidine incorporation. **C:** effect of PKA inhibitor, protein kinase A inhibitor 14-22 (PKAI), on the inhibitory effect of D₃ receptor in aortic VSMCs from SD rats. The cells were incubated with the indicated reagents: NE (10⁻⁶ M), PD (10⁻⁷ M), and PKAI (10⁻⁶ M) for 24 h. VSMC proliferation was determined by [³H]thymidine incorporation. **D:** effect of PKA activator 8-(4-chlorophenylthio)adenosine-3',5'-cyclic monophosphorothioate, Sp-isomer sodium salt (Sp-cAMP[S]; PKAa) and PKAI on NE-mediated proliferation of aortic VSMCs from SD rats. The cells were incubated with the indicated reagents: NE (10⁻⁶ M), PKAI (10⁻⁶ M), and Sp-cAMP[S] (10⁻⁷ M) for 24 h. VSMC proliferation was determined by [³H]thymidine incorporation. All results are expressed as cpm/mg protein [*n* = 9 (**A**), *n* = 5 (**B**), *n* = 8 (**C**), and *n* = 4 (**D**) experiments]. **P* < 0.05 vs. control; ***P* < 0.01 vs. control; #*P* < 0.05 vs. NE (ANOVA, Duncan's test).

Fig. 4.

A: Effect of D₁-like and D₃ receptors on NE-mediated proliferation of aortic VSMCs from SD rats. A: VSMC proliferation was determined by [^3H]thymidine incorporation after incubation with the indicated concentrations of NE (10^{-6} M) with or without the presence of PD (10^{-7} M) and Fen (10^{-7} M). Results are expressed as cpm/mg protein ($n = 8$ experiments). B: VSMC proliferation was determined by cell number after incubation with the indicated concentrations of NE (10^{-6} M) with or without the presence of PD (10^{-7} M) and Fen (10^{-7} M). Results are expressed as cell number per well ($n = 4$ experiments). C: VSMC proliferation was determined by the 3-(4,5-dimethyl-thiazol-2-yl)-diphenyltetrazolium bromide (MTT) method after incubation with the indicated concentrations of NE (10^{-6} M) with or without the presence of

PD (10^{-7} M) and Fen (10^{-7} M). Results are expressed as MTT optical density (OD) = 490 nm ($n = 14$ experiments). * $P < 0.01$ vs. control; # $P < 0.05$ vs. NE, & $P < 0.05$ vs. NE + Fen or NE + PD (ANOVA, Duncan's test).