Dopamine D1 Receptor Augmentation of D3 Receptor Action in Rat Aortic or Mesenteric Vascular Smooth Muscles

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Abstract—Dopamine is an important modulator of blood pressure, in part, by regulating vascular resistance. To test the hypothesis that D1 and D3 receptors interact in vascular smooth muscle cells, we studied A10 cells, a rat aortic smooth muscle cell line, and rat mesenteric arteries that express both dopamine receptor subtypes. Fenoldopam, a D1-like receptor agonist, increased both D1 and D3 receptor protein in a time-dependent and a concentration-dependent manner in A10 cells. The effect of fenoldopam was specific because a D1-like receptor antagonist, SCH23390 (10^-7 M/24 h), completely blocked the stimulatory effect of fenoldopam (10^-7 M/24 h) (D1 receptor: control = 21 ± 1 density units [DU]); SCH23390 = 23 ± 2 DU; fenoldopam = 33 ± 2 DU; fenoldopam + SCH23390 = 23 ± 2 DU; n = 10). D1 and D3 receptors physically interacted with each other because fenoldopam (10^-7 M/24 h) increased D1/D3 receptor coimmunoprecipitation (35 ± 5 versus 65 ± 5 DU; n = 8). A D3 receptor agonist, PD128907, relaxed mesenteric arterial rings independent of the endothelium, effects that were blocked by a D3 receptor antagonist, U99194A. Costimulation of D1 and D3 receptors led to additive vasorelaxation. We conclude that the D1 receptor regulates the D3 receptor by physical interaction and receptor expression. D3 receptor stimulation augments D1 receptor vasorelaxant effects. An interaction of D1 and D3 receptors may be involved in the regulation of blood pressure. (Hypertension. 2004; 43:673-679.)

Key Words: receptor ■ dopamine ■ arteries ■ blood pressure

Dopamine, a well known neurotransmitter in the central nervous system, has recently been characterized as an important modulator of blood pressure, sodium balance, and renal and adrenal function and is relevant to the pathogenesis and/or maintenance of hypertension.1–4 Dopamine receptors are classified into 2 families: the D1-like receptor subfamily includes the D1 and the D3 receptor, while the D2, D3, and D4 receptors belong to the D2-like subfamily. Whereas the D1-like receptors couple to the stimulatory G protein, Gs, and thus activate adenylyl cyclase, all of the D2-like receptors couple to the inhibitory G protein, Gi or Go, and inhibit adenylyl cyclase and calcium channels and modulate potassium channels.1–4

Both D1 and D3 receptors are expressed in vascular smooth muscle cells, and the activation of the D1 receptor relaxes blood vessels and decreases blood pressure.5,6 However, the effect of D1 receptors on resistance vessels is not clear. A low intravenous dose of R(+)-7-hydroxydipropyl-aminotetralin (7-OH-DPAT), a D2-like agonist with a 50-fold selectivity for the D2 over the D1 receptor, constricts postglomerular arterioles without affecting systemic blood pressure.4 A higher dose, however, decreases blood pressure.4 To test the hypothesis that D1 and D3 receptors interact in vascular smooth muscle cells, we studied the effect of fenoldopam, a D1-like receptor agonist, on D1 and D3 receptor expressions in rat thoracic aorta-derived smooth muscle cell line (A10).7 We also studied the effect of D1 and D3 receptor agonists on the wall tension of rat mesenteric arterial rings.

Methods

Cell Culture and Sample Preparation

Embryonic thoracic aortic smooth muscle cells7,8 (passage 10 to 20) from normotensive Berlin-Druckrey IX (A10; CRL 1476, ATCC) were cultured at 37 °C in 95% air/5% CO2 atmosphere in Dulbecco modified eagle medium. A10 cells (80% confluence) and mesenteric arteries from sodium pentobarbital-anesthetized Wistar-Kyoto (WKY) rats (Taconic, Germantown, NY) were homogenized in ice-cold lysis buffer (5 mL/g tissue) (20 mmol/L Tris-HCl, pH 7.4; 2 mmol/L EDTA, pH 8.0; 2 mmol/L EGTA; 100 mmol/L NaCl; 10 μg/mL leupeptin; 10 μg/mL aprotinin; 2 mmol/L phenylmethylsulfonyl fluoride; 1% NP-40), sonicated, kept on ice for 1 hour, and centrifuged at 16 000g for 30 minutes. All samples were stored at −70°C until use. All experiments were approved by the Georgetown University Animal Use and Care Committee.

Received July 16, 2003; first decision August 21, 2003; revision accepted November 20, 2003.
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Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000118958.27649.6f
Immunoblotting
The antibodies are polyclonal, purified, and antipeptides. The rat D3 receptor immunizing peptide (299-GSEEQQPPPC-307) (Research Genetics)9–12 and the rat D3 receptor immunizing peptide (228-QPP SPG QTH GGL KRY YSI C-306) are located on the third extracellular loop of their corresponding receptors.13,14 The specificity of these antibodies has been reported.9–14 A10 cells were treated with vehicle (dH2O), a D3-like receptor agonist (fenoldopam) (Sigma, St. Louis, Mo),4.5.9.11.13–17 or a D3-like receptor antagonist (SCH23390) (Sigma, St. Louis, Mo)1.1 at the indicated concentrations and times. The transblots were probed with the D3 (1:250) (Alpha Diagnostic International, San Antonio, Tex) or the D3 receptor antibody (Research Genetics)1.800. The amount of protein transferred onto the membranes was determined by Ponceau-S staining and immunoblotting for actin.

Immunoprecipitation
A10 cells were incubated with vehicle (dH2O) or fenoldopam (10−7 M) for 24 hours, as described.9.14 Equal amounts of cell lysates (200 μg protein/mL supernatant) were incubated with affinity-purified anti-D3 receptor antibodies (2 μL/mL) for 1 hour and protein G agarose at 4°C for 12 hours. The immunoprecipitates were suspended in sample buffer (2X sample buffer: 100% glycerol 2.0 mL; 0.5 mmol/L Tris-HCl 2.5 mL, pH 6.8; 10% SDS 4 mL) [final concentration=0.14 mmol/L]; 2 mg/mL bromophenol blue 0.5 mL; 2-mercaptopethanol 0.5 mL; added dH2O until total volume 10 mL), boiled for 10 minutes, and subjected to immunoblotting with the D3 receptor antibody. To determine the specificity of the bands found on the immunoblots, preimmune serum of D1 receptor antibody (negative control) and D3 receptor antibodies (positive control) were used as the immunoprecipitates instead of the D3 receptor antibodies.

Immunohistochemistry
The rat mesenteric artery, cleared of blood with ice-cold oxygenated saline and kept in Histochoice (Amresco, Solon, Ohio) for 1 to 2 days at 4°C, were sectioned (4 μm), embedded in paraffin, and mounted on slides.17 The tissue was deparaffinized and rehydrated by successive incubations in xylene, 100% ethanol, 95% ethanol, 75% ethanol, and phosphate-buffered saline. The tissue was then briefly treated with 0.1% Triton X-100, 10 mmol/L sodium citrate (pH 6.0), and blocked sequentially with 5% goat serum in phosphate-buffered saline overnight at 4°C. The tissue sections were incubated with anti-D3 receptor antibody (1:200) at room temperature for 1.5 hours. After staining with Vector VIP substrate kit, the slides were permanently mounted. The antibody (1:200) at 4°C for 12 hours. The molecular sizes are given.

Mesenteric Artery Study
A portion of intestine and mesentery removed from male WKY rats (300 to 350 g, n=12) anesthetized with sodium pentobarbital (50 mg/kg) was kept in ice-cold physiological salt solution (PSS). Four segments of third-generation resistance vessels were dissected under an operating microscope (Olympus, SZ40) and mounted as ring preparations on four 40-μm stainless-steel wires in an isometric Mulvany-Halpern small-vessel myograph (model M610M; J.P. Trading, Science Park, Aarhus, Denmark).18 One wire was attached to a force transducer and the other to a micrometer.18,19 This arrangement enabled the wall tension to be measured at a predetermined internal circumference. Both the dissection and mounting of the vessels were performed in ice-cold (4°C) PSS.

After mounting, the arterial ring was equilibrated in PSS for 1 hour at 37°C at a wall tension of 0.1 mN/mm. Based on preliminary data from >100 vessels, we confirmed that a normalized circumference (L0/L∞=0.94) results in maximal active force development. The vessels were studied at L0 in all subsequent protocols.

After testing the constrictor effects of high-potassium PSS (KPSS, 125 mmol/L) or PSS containing 10−7 M norepinephrine, the vessels were rinsed 3 times with fresh PSS and allowed to recover to baseline for 15 minutes. Maximal contraction of the vessel was then achieved with 10−4 M norepinephrine and KPSS. After reaching a plateau, relaxation was induced with 10−3 M of acetylcholine to test endothelium-dependent relaxation. Ligand-induced relaxation was assessed by cumulative addition of drugs to the rings, which were submaximally (50% to 80%) preconstricted with norepinephrine or KPSS. The receptor specificities of drug effect were determined by incubating the vessels for 30 minutes with a D3-like receptor antagonist, SCH23390 (10−6 M), or the D3 receptor antagonists, U99194 (10−6 M), and (+)-UH232 (Tocris Cookson Inc, Ellisville, Mo) (10−6 M).20,24 To investigate the mechanisms of ligand-mediated vasorelaxation, studies were performed in vessels preconstricted for 30 minutes with nifedipine (Sigma)23 or apamin (Sigma) (10−6 M; a small conductance calcium-activated K+ channel blocker)26 and charybdotoxin (Sigma) (10−6 M; a large conductance calcium-activated K+ channel blocker).26 To determine the endothelium dependence of any D3 or D1 receptor agonist-induced relaxation, vessels with or without endothelium were studied. The endothelium was removed by pulling a hair along the vessels; successful denudement of the endothelium was confirmed by an absence of relaxation by acetylcholine.27 To test any interaction between D3 or D1 receptors, PD128907 (10−10 to 10−8 M) studies were performed in vessels preconstricted for 30 minutes with fenoldopam (10−8 M). To confirm the D3 receptor-mediated vasorelaxation, we used 2 other D3 receptor ligands, 7-OH-DPAT (Sigma)20,22 (agonist), and (+)-UH23220,24 (antagonist).

Statistical Analysis
The data are expressed as mean±SEM. Comparison within groups was made by repeated measures ANOVA (paired t test when only 2 groups were compared), and comparison among groups (or t test when only 2 groups were compared) was made by factorial ANOVA with Duncan test. Corresponding periods between 2 different groups were analyzed by independent t test. Fenoldopam and PD128907 sensitivity are expressed as pED50, which is the (−log) concentration of the drug required to produce 50% of the maximum response. Relaxation responses to fenoldopam and PD128907 are expressed as a percentage decrease from the maximum contractile response. A value of P<0.05 was considered significant.

Results
Specificity of Receptor Antibodies
Specific D3 receptor (≈45 kDa) bands were found in A10 cells (Figure 1, lane 2), because they were no longer visible when the
antibodies were pre-adsorbed by the immunizing peptide. The immunoblotting patterns of the D$_3$ and D$_1$ receptors were similar to those in our previous reports.

D$_1$ Receptors Increase D$_3$ Receptor Protein Expression in A10 Cells

The D$_1$-like receptor agonist, fenoldopam, increased D$_3$ receptor protein in a concentration-dependent and time-dependent manner. The stimulatory effect at 24 hours was evident at $10^{-9}$ M (Figure 2A). The stimulatory effect of fenoldopam ($10^{-7}$ M) was noted at 16 hours and maintained for at least 24 hours (Figure 2B).

The specificity of fenoldopam as a D$_1$-like receptor agonist was determined by studying the effect of a D$_1$-like receptor antagonist, SCH23390. Consistent with the data shown in Figure 2A and 2B, fenoldopam ($10^{-7}$ M/24 h) increased D$_3$ receptor protein (control = 21±1 DU; fenoldopam = 33±2 DU; n = 10; P < 0.05). SCH23390 ($10^{-7}$ M) by itself had no effect on D$_3$ receptor protein (23±2 DU) but reversed the stimulatory effect of fenoldopam on D$_3$ receptor protein expression (fenoldopam + SCH23390 = 23±2 DU; n = 10) (Figure 2C). To determine whether there is mutual receptor interaction, A10 cells were treated with PD128907 ($10^{-10}$ M to $10^{-5}$ M) for 24 hours and immunoreactive D$_1$ receptor protein was measured. Unlike the stimulatory effect of D$_1$ receptors on D$_3$ receptor expression, we did not find any effect of D$_3$ receptors on D$_1$ receptor expression (data not shown).

D$_1$-Like Agonist Increases D$_1$ Receptor Protein Expression in A10 Cells

To investigate the effect of a D$_1$-like receptor agonist on the protein expression of its own receptor, A10 cells were incubated with fenoldopam. Immunoblots showed that fenoldopam also increased D$_1$ receptor protein in a time-dependent (2 to 30 hours) and concentration-dependent ($10^{-11}$ to $10^{-8}$ M) manner. The stimulatory effect (24 hours) was evident at $10^{-8}$ M. The stimulatory effect of fenoldopam ($10^{-7}$ M) was noted at 8 hours and maintained for at least 30 hours (Figure 3A and B).

D$_1$ Receptor Physically Interacts With the D$_3$ Receptor in A10 Cells

To determine whether there is a physical interaction between the D$_3$ and the D$_1$ receptor, D$_1$ receptors were first immunoprecipitated with anti-D$_1$ receptor antibodies, and then the immunoprecipitates were probed with anti-D$_3$ receptor antibodies. The 45 kDa band, representing the coimmunoprecipitated D$_3$ and D$_1$ receptors, was increased by a 24-hour treatment with fenoldopam ($10^{-7}$ M) (control = 35±5 DU; fenoldopam = 65±5 DU; P < 0.01, n = 8) (Figure 4).

D$_3$ Receptor Exists in Rat Mesenteric Artery

We also determined whether the D$_3$ receptor is expressed in rat mesenteric arteries. Consistent with the results in A10 cells, the D$_3$ receptor immunoblots of mesenteric artery homogenates also showed a 45-kDa band (Figure 1, lane 1). Immunohistochemistry showed D$_3$ receptors in the tunica media and intima (Figure 5). Both the immunoblotting and immunohistochemistry results were specific, because the immunoblot (Figure 1, lane 1) and immunostaining (Figure 5) were no longer visible when the antibodies were pre-adsorbed with the D$_3$ receptor immunizing peptide.

Costimulation of D$_1$ and D$_3$ Receptors Has an Additive Vasorelaxant Effect in the Rat Mesenteric Artery

Effect of Agonists

We next determined the effect of D$_1$ and D$_3$ receptor agonists on the wall tension of rat mesenteric artery rings. The D$_1$
Figure 3. Effect of fenoldopam on D_1 receptor protein expression in A10 cells. Concentration-dependence (24 hours) (A) and time course (10^{-7} M) (B) of D_1 receptor protein expression in A10 cells treated with fenoldopam. All immunoblotting results are expressed as relative DU (n=6 to 7; *P<0.05 versus control (C), ANOVA, Duncan test).

(fenoldopam) and D_1 receptor agonists (PD128907 and 7-OH-DPAT) had no vasoconstrictor effect but relaxed arterial rings preconstricted with KPSS in a dose-dependent manner; the vasorelaxant effects were evident at 10^{-6} M (fenoldopam: E_max [maximum relaxation rate] = 83% ± 11%, pED_{so} = 5 ± 0.2, n = 12; PD128907: E_max = 76% ± 6%, pED_{so} = 5 ± 0.1, n = 12; 7-OH-DPAT: E_max = 87% ± 2%, pED_{so} = 7 ± 0.2, n = 4) (Figure 6A). However, the ability of fenoldopam or PD128907 to relax vessels preconstricted with 10^{-5} M norepinephrine was markedly impaired when compared with vessels preconstricted with KPSS (pED_{so}, fenoldopam: norepinephrine = 4 ± 0.2, KPSS = 5 ± 0.2, P < 0.05; pED_{so}, PD128907: norepinephrine = 4 ± 0.2, KPSS = 5 ± 0.1, n = 12, P < 0.01).

The D_1 and D_3 receptor agonist vasorelaxant effects were endothelium-independent, because the vasorelaxant effects of fenoldopam and PD128907 were not affected by the presence or absence of the endothelium (10^{-5} M PD128907: with endothelium = 25% ± 4%, without endothelium = 24% ± 6%; 10^{-6} M fenoldopam: with endothelium = 29% ± 5%, without endothelium = 26% ± 6%, P > 0.05, n = 12) (Figure 6B and C).

Effect of Antagonists

The vasodilatory effects of D_1 and D_3 receptors were specific, because the D_1-like receptor antagonist, SCH23390 (2% ± 1%), D_3 receptor antagonists, U99194A (2% ± 0.02%) and (+)-UH232 (9% ± 3%), did not have any effect by themselves but blocked the vasorelaxant effects of their respective agonists: fenoldopam (10^{-6} M) (fenoldopam = 29% ± 5%, fenoldopam + SCH23390 = 3% ± 3%, P < 0.01, n = 12); PD128907 (10^{-6} M) (PD128907 = 24% ± 6%, PD128907 + U99194A = 4% ± 2%, P < 0.01, n = 12), 7-OH DPAT (7-OH-DPAT = 90% ± 2%, 7-OH-DPAT + U99194A = 11% ± 4%, P < 0.01, n = 4) (Figure 6B, C, and D).

Effect of combined D_1 and D_3 receptor stimulation is described herein. PD128907-induced vasorelaxation was increased in vessel preincubated with fenoldopam compared with vehicle (% relaxant at 10^{-6} M: fenoldopam = 40% ± 5%, vehicle = 24% ± 6%, n = 12, P < 0.01; pED_{so}, fenoldopam = 6 ± 0.1, vehicle = 5 ± 0.1, n = 12, P < 0.05; Figure 6E). These data indicate that D_1 and D_3 receptor have additive vasorelaxant effects.

Mechanism of D_1 and D_3 receptor-induced vasorelaxation is described herein. In norepinephrine preconstricted vessels, fenoldopam-induced and PD128907-induced vasorelaxation was increased in vessel preincubated for 30 minutes with nifedipine_25 (10^{-6} M), indicating that the decreased vasorelaxant effects of fenoldopam and PD128907 in norepinephrine-preconstricted vessels may be related to a norepinephrine-induced increase in intracellular calcium (Figure 6F and G). The vasodilatory effect of PD128907 was abrogated by apamin and charybdotoxin, indicating involvement of small-conductance and/or large-conductance calcium-activated potassium channels (Figure 6G).
Discussion

The effect of D₁ receptors on arterial vascular tone is not well understood. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries. Quinpirole, a D₃ receptor agonist with a 35-fold selectivity over the D₂ receptor, has no vascular relaxant effect on mesenteric arteries.

Figure 6. Effects of D₁ and D₃ receptor agonists in rat mesenteric arterial rings. A, Dose-dependent vasorelaxation of mesenteric arterial rings, preconstricted with KCl (125 mmol/L), by D₃ receptor agonists (7-OH-DPAT and PD128907), a D₁ receptor agonist (fenoldopam) (n=12/concentration). Specificity of D₁ receptor-mediated (B) and D₃ receptor-mediated (C) vasorelaxation in rat mesenteric arterial rings. Rat mesenteric arterial rings, preconstricted with KCl (125 mmol/L), were exposed to fenoldopam (Fen, 10⁻⁶ M) and/or SCH23390 (SCH, 10⁻⁶ M) (B), PD128907 (PD, 10⁻⁶ M), and/or U99194A (U, 10⁻⁶ M) (D₃ receptor antagonist) (C). The effect of fenoldopam or PD128907 was also determined in the arterial rings with endothelium (w/ Endo) or without endothelium (w/o Endo). *P<0.01 vs fenoldopam or PD128907 alone, n=12, ANOVA, Duncan test. D, Specificity of the D₃ receptor-mediated vasorelaxation in rat mesenteric arterial rings. Rat mesenteric arterial rings, without endothelium, preconstricted with KCl (125 mmol/L), were exposed to 7-OH-DPAT (D₃ receptor agonist) and/or (w/o)-UH232 (UH232, 10⁻⁶ M) (D₃ receptor antagonist). *P<0.01 vs 7-OH-DPAT alone, n=4, ANOVA, Duncan test). E, Dose-dependent vasorelaxation of D₁ and D₃ receptors in mesenteric arterial rings. Rat mesenteric arterial rings, preconstricted with KCl (125 mmol/L), were exposed to different concentrations of PD128907 (D₃ receptor agonist) with fenoldopam (D₁-like receptor agonist, 10⁻⁷ M) or vehicle (dH₂O) preconstriction (n=12/concentration, *P<0.01 vs vehicle, Duncan test). F, Dose-dependent vasorelaxation of fenoldopam in mesenteric arterial rings preincubated with nifedipine (10⁻⁶ M) for 30 minutes. Rat mesenteric arterial rings, preconstricted with norepinephrine (10⁻⁶ M), were exposed to different concentrations of fenoldopam (D₁-like receptor agonist, 10⁻³ M to 10⁻¹⁰ M) (n=4/concentration, *P<0.05 vs vehicle, Duncan test). G, Dose-dependent vasorelaxation of PD128907 in mesenteric arterial rings preincubated with nifedipine (10⁻⁶ M), apamin (10⁻⁶ M), and charybotoxin (10⁻⁶ M) for 30 minutes. Rat mesenteric arterial rings, preconstricted with norepinephrine (10⁻⁶ M), were exposed to different concentrations of PD128907 (D₃ receptor agonist, 10⁻⁶ M to 10⁻¹⁰ M) (n=4/concentration, *P<0.05 vs vehicle, Duncan test).
cause vasorelaxation. As compared with vessels preconstricted with norepinephrine, the D1 and D3 vasorelaxation is greater in vessels preconstricted with potassium chloride. The ability of norepinephrine to impair the vasodilatory effects of dopamine has been reported. The decreased vasodilator effect of D3 agonists in vessels preconstricted with norepinephrine may be caused by an increase of intracellular calcium by activation of $\alpha_1$-adrenergic receptors. Our studies show that the vasorelaxant effects of fenoldopam (D1 receptor agonist) and PD128907 (D3 receptor agonist) are enhanced by calcium channel blockade with nifedipine. The vasodilatory effect of D1 receptors may also involve potassium channels (small-conductance and/or large-conductance calcium activated potassium). Additionally, norepinephrine can also compete with the agonists for dopamine receptor occupancy.

The vasorelaxant effect of dopamine in the rabbit pulmonary artery has been reported to be endothelium-dependent and endothelium-independent. However, in our studies, despite the presence of D1 receptors in the intima, the vasodilatory effects of the D1 and D3 receptor agonists are endothelium independent. The function of the D1 receptor in endothelial cells is not clear, but D1 receptors have cell-protective properties.

The simultaneous stimulation of D1 and D3 receptors causes a vasorelaxation that is additive rather than synergistic. This may indicate that D1 and D3 receptors induce vasorelaxation by different mechanisms. Our studies also indicate that the vasorelaxation of the mesenteric artery is probably caused by activation of postjunctional rather than prejunctional dopamine receptors, because neither D1 receptor nor D3 receptor is expressed in the tunica adventitia, where the nerve supply terminates. The reason for the inability of others to demonstrate D3 receptors in vascular smooth muscles is not clear.

We find that D1 and D3 receptors coimmunoprecipitate, and that coimmunoprecipitation is increased by D1 receptor stimulation. There are 2 possible explanations for this observation: increased D1 and D3 receptor expression, per se, or an increased physical interaction between D1 and D3 receptors. This interaction occurs only after several hours of treatment and may explain the absence of synergism in the acute studies in mesenteric vessels. Further studies are needed to determine whether the increased interaction between these 2 receptors is a direct or an indirect mechanism. However, homooligomerization or heterooligomerization of several G protein-coupled receptors have been reported.

In summary, we have demonstrated that the D1 receptor regulates the D3 receptor by physical interaction and receptor expression. D1 receptor stimulation augments D3 receptor vasodilatory effects. A D1/D3 receptor interaction may be involved in the regulation of blood pressure.

**Perspectives**

The vasodilator effect of dopamine via D1-like receptors is mediated mainly by cAMP/protein kinase A. It seems paradoxical that the D1 receptor dilates the mesenteric artery because D1-like receptors decrease while D3-like receptors increase cAMP production. D1 receptors linkage to adenylyl cyclase inhibition is weak, unless adenylyl cyclase V is present. Adenylyl cyclase isoform V has been shown in rat pulmonary arterial smooth muscles but not in aortic smooth muscles. Therefore, it is likely that the D3 receptor-mediated vasorelaxation in vascular smooth muscle cells is caused by its ability to stimulate K+ channels. Under these conditions, the vasorelaxant effects of D1-like receptors caused by cAMP/PKA may be additive to the vasorelaxant effect of D3 receptors caused by stimulation of K+ channels.

**Acknowledgments**

These studies were supported in part by grants from the National Institutes of Health, HL 074940, HL 23081, DK 39308, HL06866, DK52612, and HL62211.

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Hypertension. 2004;43:673-679; originally published online February 9, 2004; doi: 10.1161/01.HYP.0000118958.27649.6f

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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