Dopamine $D_1$ Receptor Augmentation of $D_3$ 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 $D_1$ and $D_3$ 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 $D_1$-like receptor agonist, increased both $D_1$ and $D_3$ receptor protein in a time-dependent and a concentration-dependent manner in A10 cells. The effect of fenoldopam was specific because a $D_1$-like receptor antagonist, SCH23390 (10$^{-7}$ M/24 h), completely blocked the stimulatory effect of fenoldopam (10$^{-7}$ M/24 h) ($D_1$ receptor: control=21±1 density units [DU]); SCH23390=23±2 DU; fenoldopam=33±2 DU; fenoldopam+SCH23390=23±2 DU; n=10). $D_1$ and $D_3$ receptors physically interacted with each other because fenoldopam (10$^{-7}$ M/24 h) increased $D_1/D_3$ receptor coimmunoprecipitation (35±5 versus 65±5 DU; n=8). A $D_3$ receptor agonist, PD128907, relaxed mesenteric arterial rings independent of the endothelium, effects that were blocked by a $D_3$ receptor antagonist, U99194A. Costimulation of $D_1$ and $D_3$ receptors led to additive vasorelaxation. We conclude that the $D_1$ receptor regulates the $D_3$ receptor by physical interaction and receptor expression. $D_1$ receptor stimulation augments $D_3$ receptor vasorelaxant effects. An interaction of $D_1$ and $D_3$ 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 $D_1$-like receptor subfamily includes the $D_1$ and the $D_3$ receptor, while the $D_2$, $D_3$, and $D_4$ receptors belong to the $D_2$-like subfamily. Whereas the $D_1$-like receptors couple to the stimulatory G protein, $G_S$, and thus activate adenylyl cyclase, all of the $D_2$-like receptors couple to the inhibitory G protein, $G_I/G_Q$, and inhibit adenylyl cyclase and calcium channels and modulate potassium channels.1–4

Both $D_1$ and $D_3$ receptors are expressed in vascular smooth muscle cells, and the activation of the $D_1$ receptor relaxes blood vessels and decreases blood pressure.5,6 However, the effect of $D_3$ receptors on resistance vessels is not clear. A low intravenous dose of R(+)-7-hydroxydipropylaminotetralin (7-OH-DPAT), a $D_3$-like agonist with a 50-fold selectivity for the $D_3$ over the $D_1$ receptor, constricts postglomerular arterioles without affecting systemic blood pressure. A higher dose, however, decreases blood pressure.4 To test the hypothesis that $D_1$ and $D_3$ receptors interact in vascular smooth muscle cells, we studied the effect of fenoldopam, a $D_1$-like receptor agonist, on $D_1$ and $D_3$ receptor expressions in rat thoracic aorta-derived smooth muscle cell line (A10).7 We also studied the effect of $D_1$ and $D_3$ 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% CO$_2$ 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.

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Immunoblotting

The antibodies are polyclonal, purified, and antipeptides. The rat D1 receptor immunizing peptide (299-GSEEETQPFCC-307) (Research Genetics)9-12 and the rat D3 receptor immunizing peptide (288-QPPPSPG 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 D1-like receptor agonist (fenoldopam) (Sigma, St. Louis, Mo),9-11,13,15 or a D1-like receptor antagonist (SCH23390) (Sigma, St. Louis, Mo)9,11 at the indicated concentrations and times. The transblots were probed with the D1 (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 α-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-D1 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-mercaptoethanol 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 D1 receptor antibodies.

Immunohistochemistry

The rat mesenteric artery, cleared of blood with ice-cold oxygenated saline and kept in Histochrome (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 50% ethanol. The tissue was dehydrated in a 100% ethanol bath at room temperature for 1.5 hours. After staining with Vector VIP substrate kit, the slides were permanently mounted. The data are expressed as mean ±SEM. Comparison within groups was made by repeated measures ANOVA (or 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.

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 for KPSS (10⁻⁵ M), the internal circumference (L0) of 0.9 Lmax 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 10⁻⁵ M norepinephrine, the vessels were rinsed 3 times with fresh PSS and allowed to recover to baseline for 15 minutes. Maximal constriction of the vessel was then achieved with 10⁻⁵ M norepinephrine and KPSS. After reaching a plateau, relaxation was induced with 10⁻⁵ 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%) precontracted with norepinephrine or KPSS. To test the action of D1 and/or D3 receptors, cumulative concentrations (10⁻⁶ to 10⁻⁶ M) of a D1-like receptor agonist, fenoldopam, and/or D3 receptor agonist, PD128907 (Sigma)20,21 and 7-OH-DPAT (Sigma)22,23 were added to the arterial rings preconstricted with norepinephrine or KPSS. The receptor specificities of drug effect were determined by incubating the vessels for 30 minutes with a D1-like antagonist, SCH23390 (10⁻⁶ M), or the D3 receptor antagonists, U99194 (10⁻⁶ M)23 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 precontracted for 30 minutes with nifedipine (Sigma)25 or apamin (Sigma) (10⁻⁶ M: a small conductance calcium-activated K⁺ channel blocker)26 and charybdotoxin (Sigma) (10⁻⁸ M: a large conductance calcium-activated K⁺ channel blocker).26 To determine the endothelium dependence of any D1 or D3 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 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

Results

Specificity of Receptor Antibodies

Specific D1 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 D3 and D1 receptors were similar to those in our previous reports.9,11,13,14,17

D1 Receptors Increase D3 Receptor Protein Expression in A10 Cells

The D1-like receptor agonist, fenoldopam, increased D3 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 D1-like receptor agonist was determined by studying the effect of a D1-like receptor antagonist, SCH23390. Consistent with the data shown in Figure 2A and 2B, fenoldopam (10⁻⁷ M/24 h) increased D1 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 D1 receptor protein (23±2 DU) but reversed the stimulatory effect of fenoldopam on D3 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 D1 receptor protein was measured. Unlike the stimulatory effect of D1 receptors on D3 receptor expression, we did not find any effect of D3 receptors on D1 receptor expression (data not shown).

D1-Like Agonist Increases D1 Receptor Protein Expression in A10 Cells

To investigate the effect of a D1-like receptor agonist on the protein expression of its own receptor, A10 cells were incubated with fenoldopam. Immunoblots showed that fenoldopam also increased D1 receptor protein in a time-dependent (2 to 30 hours) and concentration-dependent (10⁻¹¹ to 10⁻⁵ 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).

D1 Receptor Physically Interacts With the D3 Receptor in A10 Cells

To determine whether there is a physical interaction between the D3 and the D1 receptor, D1 receptors were first immunoprecipitated with anti-D1 receptor antibodies, and then the immunoprecipitates were probed with anti-D3 receptor antibodies. The 45 kDa band, representing the coimmunoprecipitated D3 and D1 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).

D3 Receptor Exists in Rat Mesenteric Artery

We also determined whether the D3 receptor is expressed in rat mesenteric arteries. Consistent with the results in A10 cells, the D3 receptor immunoblots of mesenteric artery homogenates also showed a 45-kDa band (Figure 1, lane 1). Immunohistochemistry showed D3 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 D3 receptor immunizing peptide.

Costimulation of D1 and D3 Receptors Has an Additive Vasorelaxant Effect in the Rat Mesenteric Artery

Effect of Agonists

We next determined the effect of D1 and D3 receptor agonists on the wall tension of rat mesenteric artery rings. The D1,
fenoldopam: norepinephrine = 4 ± 0.2, KPSS = 5 ± 0.2, P < 0.05; pED50: PD128907: norepinephrine = 4 ± 0.2, KPSS = 5 ± 0.1, n = 12, P < 0.01).

The D1 and D3 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 (10−6 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 vasorelaxant effects of D1 and D3 receptors were specific, because the D1-like receptor antagonist, SCH23390 (2% ± 1%), D1 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 D1 and D3 receptor stimulation is described herein. PD128907-induced vasorelaxation was increased in vessel preincubated with fenoldopam compared with vehicle (% relaxation at 10−6 M: fenoldopam = 40% ± 5%, vehicle = 24% ± 6%, n = 12, P < 0.01; pED50: fenoldopam = 6 ± 0.1, vehicle = 5 ± 0.1, n = 12, P < 0.05; Figure 6E). These data indicate that D1 and D3 receptor have additive vasorelaxant effects.

Mechanism of D1 and D3 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 nifedipine25 (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 D_1 receptors on arterial vascular tone is not well understood. Quinpirole, a D_3 receptor agonist with a 35-fold selectivity over the D_2 receptor, has no vascular relaxant effect on mesenteric arteries. Another D_3 receptor agonist, pramipexole, with a 60 selectivity over the D_2 receptor, decreases vascular resistance, although part of the effect could be accounted for by an interaction with the D_1 receptor.

In anesthetized rats, the systemic infusion of 7-OH-DPAT, a D_3 receptor agonist with a 50-fold selectivity over the D_2 receptor, decreases renal blood flow by postglomerular constriction, resulting in an increase in glomerular filtration rate. In conscious dogs, the intrarenal arterial infusion of quinpirole also decreases renal blood flow, but glomerular filtration rate is decreased as well. The reason for these apparent discrepancies is not clear. However, the effect of D_2-like receptors on vascular tone may vary depending on the resisting tone and the arterial segment being studied. For example, D_1 receptor agonist increases the vascular tone of the rat tail artery. In other vessels, including the renal artery, D_1 receptors are vasodilatory. Prejunctional D_2-like receptors are vasodilatory whereas postjunctional D_2-like receptors can induce vasoconstriction when resting vascular tone is low or vasodilation when resting vascular tone is high.

Our studies show that in the maximally vasorelaxed mesenteric artery, 2 different D_3 receptor agonists, PD128907, with a 120-fold selectivity over the D_2 receptor, and 7-OH-DPAT, have no effect on vascular contractility. However, when the mesenteric rings are preconstricted, the PD128907, 7-OH-DPAT, and fenoldopam, by themselves,
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.³³ The decreased vasodilator effect of D₃ agonists in vessels preconstricted with norepinephrine may be caused by an increase of intracellular calcium by activation of α₁-adrenergic receptors.³⁴⁻³⁵ 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 D₃ receptors may also involve potassium channels (small-conductance and/or large-conductance calcium activated potassium).³⁶,³⁷ Additionally, norepinephrine can also compete with the agonists for dopamine receptor occupancy.³⁸,³⁹

The vasorelaxant effect of dopamine in the rabbit pulmonary artery has been reported to be endothelium-dependent and endothelium-independent.⁴⁰ 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 D₃ receptor in endothelial cells is not clear, but D₃ receptors have cell-protective properties.⁴¹ In human brain endothelial cells, the D₃ agonist, PD128907, inhibits superoxide production, determined by cytochrome C reduction test,⁴² an effect that is blocked by a D₃ antagonist, U99194A, which by itself does not have any effect (Z. Yang, C. Zeng, K.S. Kim, P.A. Jose, unpublished data, 2003).

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.⁴₄

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 D₃ and D₁ 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 hetero-oligomerization of several G protein-coupled receptors have been reported.⁴⁵⁻⁴⁶

In summary, we have demonstrated that the D₁ receptor regulates the D₃ receptor by physical interaction and receptor expression, D₃ receptor stimulation augments D₁ 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.⁴⁷,⁴⁸ It seems paradoxical that the D₃ receptor dilates the mesenteric artery because D₃-like receptors decrease while D₁-like receptors increase cAMP production.¹⁻³ D₁ receptors linkage to adenyl cyclase inhibition is weak, unless adenyl cyclase V is present.⁴⁹ Adenyl cyclase isofrom V has been shown in rat pulmonary arterial smooth muscles but not in aortic smooth muscles.⁵⁰ Therefore, it is likely that the D₁ receptor-mediated vasorelaxation in vascular smooth muscle cells is caused by its ability to stimulate K⁺ channels.⁵¹ 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.³⁶,³⁷

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**References**


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