Aberrant D₁ and D₃ Dopamine Receptor Transregulation in Hypertension

Chunyu Zeng, Dan Wang, Laureano D. Asico, William J. Welch, Christopher S. Wilcox, Ulrich Hopfer, Gilbert M. Eisner, Robin A. Felder, Pedro A. Jose

Abstract—Dopamine plays a role in the regulation of blood pressure by inhibition of sodium transport in renal proximal tubules (RPTs) and relaxation of vascular smooth muscles. Because dopamine receptors can regulate and interact with each other, we studied the interaction of D₁ and D₃ receptors in immortalized RPT cells and mesenteric arteries from Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHRs), and in human coronary artery smooth muscle cells (CASMCs). In WKY rats, the D₁-like agonist, fenoldopam, increased D₃ receptor protein in a time-dependent and concentration-dependent manner (EC₅₀=4.5×10⁻⁹ M, t₁/₂=15.8 hours). In SHRs, fenoldopam (10⁻⁵ M) actually decreased the expression of D₁ receptors. D₁ and D₃ receptor co-immunoprecipitation was increased by fenoldopam (10⁻⁷ M/24 h) in WKY rats but not in SHRs. The effects of fenoldopam in CASMCs were similar as those in WKY RPT cells (ie, fenoldopam increased D₁ and D₃ receptor proteins). Both D₃ (PD128907, Emax=80%±6%, pED₅₀=5±0.1) and D₁-like receptor (fenoldopam, Emax=81%±8%, pED₅₀=5±0.2, n=12) agonists relaxed mesenteric arterial rings. Co-stimulation of D₁ and D₃ receptors led to additive vasorelaxation in WKY rats, but not in SHRs. D₁ and D₃ receptors interact differently in WKY and SHRs. Altered interactions between D₁ and D₃ receptors may play a role in the pathogenesis of genetic hypertension, including human hypertension, because these receptors also interact in human vascular smooth muscle cells. (Hypertension. 2004;43:654-660.)

Key Words: receptors ■ dopamine ■ rats ■ hypertension ■ normotension ■ kidney ■ vascular smooth muscle

Several studies have reported that an impaired renal dopaminergic system may contribute to the pathogenesis of hypertension.¹² There is increasing evidence for a direct interaction between D₁-like and D₃-like receptors in the kidney. In vitro studies have shown that a D₃-like receptor, in concert with a D₁-like receptor, synergistically decreases Na⁺-K⁺-ATPase, sodium-phosphate co-transporter, and sodium hydrogen exchanger activities in renal proximal tubule and other cells.³⁻⁵ In rats, during conditions of normal sodium load, and especially with increased sodium load, D₁-like and D₃-like receptors interact to increase sodium excretion.¹²⁻⁶⁻⁷

The D₁-like receptor interacting with the specific D₃-like receptor subtype in renal sodium transport is unknown. The D₁-like receptor subfamily includes the D₁ and the D₅ receptors, whereas the D₂, D₃, and D₄ receptors belong to the D₂-like receptor subfamily.¹² Although D₂ and D₃ receptors are expressed in renal proximal tubules, the major D₂-like receptor expressed in rat renal proximal tubules is the D₂ receptor.⁸⁻⁹ D₁ and D₃ receptors are expressed in renal proximal tubules, but the increase in cAMP production secondary to D₁-like agonist stimulation is primarily a D₁ rather than a D₃ receptor effect.¹⁰ There are no studies on the interaction between D₃ and D₅ receptors, but an interaction between D₁ and D₅ receptors has been reported. In the medulloblastoma TE671 cell line endogenously expressing only D₁ and D₃ dopamine receptors and not the other dopamine receptor subtypes, stimulation of the D₁ receptor increases D₃ receptor mRNA levels.¹¹ In a previous study, we showed that D₁ and D₃ receptors co-localized in immortalized renal proximal tubule (RPT) cells from normotensive Wistar-Kyoto (WKY) rats and that activation of D₁ dopamine receptors increased D₃ receptor protein expression in these cells.¹² We have found that these immortalized RPT cells have characteristics similar to freshly obtained RPTs and RPT brush-border membranes, at least regarding D₁-like receptors and responses to G-protein stimulation.¹³⁻¹⁵ The present studies were designed to determine whether D₁-like receptors regulate D₃ and D₅ receptors and whether the regulation is different in RPT cells from WKY rats and spontaneously hypertensive rats (SHRs). To determine whether D₁ and D₃ receptors also interact in vascular smooth muscle cells, we studied the effect of D₁ and D₃ receptor

Received September 2, 2003; first decision, September 30, 2003; revision accepted December 1, 2003.

From the Departments of Pediatrics (C.Z., L.D.A., G.M.E., P.A.J.), Physiology and Biophysics (P.A.J.), and Internal Medicine (G.M.E.) and Division of Nephrology and Hypertension and Center for Hypertension and Renal Disease Research (D.W., W.J.W., C.S.W.), Georgetown University Medical Center, Washington, DC; Department of Physiology (U.H.), Case Western Reserve School of Medicine, Cleveland, Oh; Department of Pathology (R.A.F.), Virginia University for the Health Sciences, Charlottesville; and Department of Cardiology (C.Z.), Daping Hospital, Third Military Medical University, Chongqing, People’s Republic of China.

Correspondence to Dr Chunyu Zeng, Department of Pediatrics, PHC-2, Georgetown University Medical Center, 3800 Reservoir Road, NW, Washington, DC 20007. E-mail cyzeng1@hotmail.com

© 2004 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000114601.30306.bf
agonists on the wall tension of rat mesenteric arterial rings from WKY rats and SHRs. We also studied the effect of fenoldopam, a D1-like receptor agonist, on D1 and D3 receptor expression in human coronary vascular smooth muscle cells (CASMCs).16

Methods

Cell Culture

Immortalized RPT cells from WKY rats and SHRs were cultured at 37°C in 95% air/5% CO2 atmosphere in DMEM/F-12 culture media, as previously described;17 CASMCs (from Cambrex Bio Science Walkersville, Walkersville, Md) were cultured in smooth muscle growth medium-3.16 The cells (80% confluence) were extracted in ice-cold lysis buffer (phosphate-buffered saline with 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L PMSF, 10 µg/mL aprotinin, and 10 µg/mL leupeptin), sonicated, kept on ice for 1 hour, and centrifuged at 16 000g for 30 minutes. The supernatants were stored at −70°C until use for immunoblotting and/or immunoprecipitation.

Immunoblotting

Rat RPT cells and CASMCs were treated with vehicle (dH2O), a D1-like receptor agonist (fenoldopam), or a D1-like receptor antagonist (SCH23390) at the indicated concentrations and times. Immunoblotting was performed as previously reported, except that the transblots were probed with the D1 receptor antibody (1:800) or the D3 receptor antibody (1:250).13,17,19 The amount of protein transferred onto the membranes was determined by Ponceau-S staining and immunoblotting for α-actin. The receptor densities were normalized by α-actin.

Immunoprecipitation

RPT cells were incubated with vehicle or fenoldopam (10−7 M or 10−8 M) for 30 minutes and 24 hours, as described.13,17 The cells were lysed with ice-cold lysis buffer for 1 hour and centrifuged at 16 000g for 30 minutes. Equal amounts of lysates (500 µg protein/mL supernatant) were incubated with anti-D1 receptor antibody (2 µL/mL) for 1 hour and protein-G agarose at 4°C for 12 hours. The immunoprecipitates were pelleted and washed four times with lysis buffer. The pellets were suspended in sample buffer, boiled for 10 minutes, and subjected to immunoblotting with the D1 receptor antibody. To determine the specificity of the bands, pre-immune serum of D1 receptor antibody (negative control) and D1 receptor antibody (positive control) were used as immunoprecipitants, instead of the D1 receptor antibody. The density of the bands was quantified by densitometry, using Quantscan (Ferguson, MO), as previously reported.13,17,19

Mesenteric Artery Study

Male WKY rats and SHRs (Taconic, Germanton, NY) (300 to 350 g, n = 12 rats/group) anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal injection), underwent laparotomy, and a portion of intestine and mesentery was cut and kept in ice-cold physiological salt solution (PSS) for isolation of resistance vessels. Four segments of third-generation resistance vessels (250 to 20 µm) were mounted as ring preparations on four 40–m stainless-steel wires in an isometric Mulvaney-Halpern small-vessel myograph (model M610M, J.P. Trading, Science Park, Aarhus, Denmark).20 In some vessels, the endothelium was removed by pulling a hair along the vessels; successful denudement of the endothelium was confirmed by an absence of relaxation with the addition of acetylcholine.21 One wire was attached to a force transducer and the other to a micrometer.21,22 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 mesenteric artery 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) = 0.9 Lmax results in maximal active force development. The vessels were studied at L0 in all subsequent protocols. The vessels were stimulated by high-potassium (125 mmol/L) PSS (KPSS). After completion of the activation procedure, the vessels were rinsed three times with fresh PSS and allowed to recover to baseline for 15 minutes. Maximal contraction of the vessel was achieved with KPSS. After reaching a plateau, relaxation was induced with 10−5 M of acetylcholine, which tests for endothelium-dependent relaxation. Ligand-induced relaxation was assessed by cumulative addition of drugs to the rings, which were submaximally (50% to 80%) pre-constricted with KPSS. KPSS contracted the mesenteric arteries to the same degree in WKY rats and SHRs (67% ± 8% versus 66% ± 10%, n = 12, P = NS).

To test the action of D1 and/or D3 receptors on the mesenteric arterioles, cumulative concentrations (10−9 M to 10−3 M) of a D1-like receptor agonist, fenoldopam, and/or a D3 receptor agonist, PD128907, were added to the arterial rings pre-constricted by KPSS. To test the interaction between D1 or D3 receptor, fenoldopam-induced (10−9 to 10−3 M) relaxation was studied in vessels pre-incubated for 30 minutes with PD128907 (10−6 M).

Materials

Rabbit anti-rat D1 receptor antibody (D3R12A) was produced against a synthetic oligopeptide from the amino acid sequence of rat D1 receptor (amino acids 288 to 306, QPPSPG QTHGGLRKYYSIC) (Alpha Diagnostic International, San Antonio, Tex). Rabbit anti-rat D1 receptor antibody was produced against a synthetic oligopeptide from the amino acid sequence of rat D1 receptor (amino acids 299 to 307, GSETETQFP) (Research Genetics). We have reported the specificity of the D1 and the D3 receptor antibodies.13,17,19 Fenoldopam, SCH23390, U99194A, and PD128907 were from Sigma (St. Louis, Mo). Other chemicals for various buffers were of the highest purity available and purchased from Sigma or Gibco. Mouse monoclonal α-actin antibody is from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif).

Statistical Analysis

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 sensitivities in blood vessels are expressed as pED50, which is the (−log) concentration of drug required to produce 50% of the maximum response. Relaxation responses to the fenoldopam and PD128907 are expressed as a percentage decrease from the maximum contractile response. A value of P < 0.05 was considered significant.

Results

Fenoldopam Increased D3 Receptor Expression in Rat RPT Cells but not in SHRs

Fenoldopam increased D3 receptor expression in a concentration-dependent and time-dependent manner in RPT cells from WKY rats. The stimulatory effect was evident at 10−9 M with a 50% increase (EC50 = 4.5 × 10−9 M) in D3 receptor expression. The stimulatory effect of fenoldopam (10−7 M) was noted as early as 8 hours and maintained for at least 30 hours (t1/2 = 15.8 h). In RPT cells from SHRs, fenoldopam (10−7 M) had no effect on D3 receptor expression (WKY rats: control = 1.1 ± 0.04, fenoldopam = 1.5 ± 0.07; SHRs: control = 0.8 ± 0.05, fenoldopam = 0.7 ± 0.05; n = 11/group) (Figure 1A). To determine whether higher concentrations of fenoldopam could have any effect on D3 receptor expression, SHR cells were treated with varying concentrations of fenoldopam for 24 hours. Consistent with the results...
from Figure 1A, $10^{-6}$ to $10^{-10}$ M fenoldopam had no effect on D$_3$ receptor protein expression, with $10^{-5}$ M fenoldopam actually decreasing it (Figure 1B).

The specificity of fenoldopam as a D$_1$-like receptor agonist was determined by studying the effect of the D$_1$-like receptor antagonist, SCH23390 in WKY RPT cells. Consistent with the current and previous results from aortic vascular smooth muscle cells, fenoldopam (10$^{-7}$ M) for 24 hours (A), with fenoldopam (10$^{-7}$ M) for 30 minutes in WKY rats and SHRs or fenoldopam (10$^{-6}$ M) for 30 minutes in SHR cells (B). Thereafter, the samples were immunoprecipitated with anti-D$_3$ receptor antibodies and immunoblotted with anti-D$_3$ receptor antibodies ($P<0.05$ versus control, n=7 to 9, ANOVA, Duncan test). One immunoblot (45 kDa) is depicted in the inset: (lane 1 = positive control, lane 2 = negative control, lane 3 = vehicle-treated RPT cells of WKY rats, lane 4 = fenoldopam-treated RPT cells of WKY rats, lane 5 = vehicle-treated RPT cells of SHRs, lane 6 = fenoldopam-treated RPT cells of SHRs). For a positive control, anti-D$_3$ antibodies (10$^{-9}$ g/mL) were used as the immunoprecipitant; for a negative control, pre-immune rabbit serum (20 L/mL) was used as the immunoprecipitant instead of the anti-D$_1$ receptor antibodies, and anti-D$_3$ receptor antibodies were used for immunoblotting as described.
**D1 Receptor Co-immunoprecipitated With the D3 Receptor in Rat RPT Cells**

To determine whether there is a physical interaction between the D1 and the D3 receptor, D1 receptors were first immunoprecipitated with anti-D1 receptor antibodies and then probed with anti-D3 receptor antibodies. As shown in Figure 2A, the 45-kDa band, representing the co-immunoprecipitated D3 and D1 receptors, was increased by a 24-hour treatment of fenoldopam (10^-7 M) in RPT cells from WKY rats, consistent with our previous report in aortic vascular smooth muscle cells. However, no effect was observed in SHRs (WKY: control=25±4 DU, fenoldopam=40±3 DU; n=7, P<0.05; SHRs: control=18±3 DU, fenoldopam=17±3 DU; n=7, P=NS).

To investigate whether short-term stimulation can affect D1/ETB receptor co-immunoprecipitation in WKY cells, and whether higher concentrations of fenoldopam had any effect on this linkage in SHR cells, WKY cells were treated with fenoldopam (10^-7 M) for 30 minutes and SHR cells were treated with different concentrations of fenoldopam (10^-7 M and 10^-6 M) for 30 minutes. Consistent with Figure 2A, fenoldopam (10^-7 M) also increased the co-immunoprecipitation of D1 and D3 receptors at 30 minutes in WKY but minimally in SHR cells. At 10^-6 M, fenoldopam increased the co-immunoprecipitation of D1 and D3 receptors in SHRs to an extent similar to that noted at 10^-7 M in WKY cells (Figure 2B). These data indicated that the sensitivities of SHRs to D1-like receptor stimulation was decreased, in agreement with previous studies from our laboratory, using cAMP as the outcome variable.1,24

**D1 Receptors Increased D3 and D1 Receptor Protein Expressions in CASMCs**

Besides the stimulatory effect of fenoldopam on D1 receptor, our previous study also showed that fenoldopam increased the expression of its own receptor (D1 receptor) in WKY RPT cells. D1 receptor regulation of D1 and D3 receptors also occurs in vascular smooth muscle cells; fenoldopam (10^-7 M/24 h) also increased D3 receptor protein in CASMCs (D1 receptor: control=0.9±0.1, fenoldopam=1.4±0.1, n=10; P<0.05). The stimulatory effect on D1 receptors in CASMCs was modest (D1 receptor: control=1±0.06, fenoldopam=1.4±0.1, n=10), compared with the effect in WKY RPT cells. The actions of fenoldopam were exerted at the D1 receptor because the effects were blocked by a D1-like receptor antagonist, SCH23390 (10^-7 M), which by itself had no effect on D1 or D3 receptor protein expression (D1 receptor: 0.9±0.1; D3 receptor: 0.9±0.1) but reversed the stimulatory effect of fenoldopam on D1 and D3 receptor protein expression (D1 receptor: 0.9±0.1; D3 receptor: 0.9±0.1, n=10) (Figure 3A and B).

**Costimulation of D1 and D3 Receptors Had an Additive Vasorelaxant Effect in the Rat Mesenteric Artery From WKY Rats but not From SHRs**

We next determined the effect of D1 and D3 receptor agonists on the wall tension of rat mesenteric artery rings. In vessels from either WKY rats or SHRs, neither D1 nor D3 receptor agonists (fenoldopam1,2 and PD128907,26 respectively) had any vasoconstrictor effect. However, in vessels from WKY rats, both agonists relaxed arterial rings pre-constricted with KPSS in a dose-dependent manner (Emax and pED50; Table); the vasorelaxant effects were evident at 10^-6 M (Figure 4A and B). These effects were specific, because the D1-like receptor antagonist, SCH23390, and D3 receptor antagonist, U99194A, did not have any effect by themselves but blocked the vasorelaxant effects of their respective agonists. There was an interaction between D1 and D3 receptors because fenoldopam-induced vasorelaxation was greater in vessels preincubated with PD128907 than in vehicle-treated vessels (Table, Figure 4C).

In SHRs, PD12897 dose-dependently relaxed mesenteric arteries pre-constricted by KPSS (Table); the effect was...
similar to that in WKY rats except for a lesser vasorelaxant effect at the two highest concentrations of the drug (Figure 4A). In contrast, the mesenteric arteries from SHRs were less sensitive and less reactive than WKY rats to the vasorelaxant effect of fenoldopam (Table and Figure 4B). Whereas pretreatment of mesenteric arteries with PD128907 enhanced the vasorelaxant effect of fenoldopam in WKY rats (Figure 4C), no additional vasorelaxant effect was noted in SHRs (Table, Figure 4D).

Discussion

We have reported a physical interaction between D₁ and D₃ receptors in rat A10 aortic smooth muscle cells.²³ In A10 and RPT cells from normotensive rats, D₁ receptor stimulation with a D₁-like agonist, fenoldopam, increases D₁ receptor protein expression.²³,²⁵ In contrast, in RPT cells from SHRs, fenoldopam has no effect of D₁ receptor expression.²³ The current study extends our previous reports and has several novel observations. First, fenoldopam, via D₁-like receptors, increases D₃ receptor protein expression in RPT cells from WKY rats. Second, in these cells, D₁ and D₃ receptors co-immunoprecipitate, which is increased by D₁-like receptor stimulation. Third, the aforementioned effects of D₁-like receptor stimulation on D₁ receptor protein and D₁/D₃ receptor co-immunoprecipitation are impaired in SHRs. Fourth, the D₁-like receptor agonist, fenoldopam, increases D₁ and D₃ receptor protein expressions in human CASMCs. Fifth, activation of either D₁ or D₃ receptor relaxes the mesenteric artery to a greater extent in WKY rats than in SHRs; neither receptor has any vasoconstrictive effect. The simultaneous stimulation of D₁ and D₃ receptors results in an additive vasorelaxation in WKY rats but not in SHRs.

D₁-like and D₃-like receptors in the kidney have been found to regulate renal sodium handling. The D₁-like receptors are always associated with a diuretic and natriuretic action.¹,² In contrast, the effect of stimulation of D₂-like receptors, independent of D₁-like receptors, on sodium excretion has produced natriuresis, no effect, or antinatriuresis in different studies.¹ However, in normotensive rats, stimulation of renal D₁-like and D₂-like receptors produces a natriuresis that is greater than that observed with D₁-like receptors alone.⁶ In addition, the natriuretic effect of endogenous renal dopamine requires activation of D₁-like and D₂-like receptors.⁷ Moreover, in concert with a D₁-like agonist, a D₂-like agonist acts synergistically to inhibit Na⁺-K⁺-ATPase and sodium hydrogen exchanger activity in RPT cells and brain striatal cells.

![Figure 4](https://example.com/figure4.png)

Figure 4. Dose-dependent vasorelaxation by D₁ and D₃ receptors in mesenteric arterial rings of WKY rats and SHRs. Rat mesenteric arterial rings, pre-constricted with KCl (125 mmol/L), were exposed to different concentrations of PD128907 (D₁ receptor agonist; A), fenoldopam (D₁-like receptor agonist; B), or fenoldopam in mesenteric arterial rings pretreated with PD128907 (10⁻⁶ M) or vehicle in WKY rats (C) and SHRs (D) (n=12/concentration, *P<0.01 versus WKY; †P<0.01 versus others; Emax=maximum relaxation induced by acetylcholine.)

### Table 1: Vasorelaxant Effect of the D₁ and the D₃ Agonist in 3rd Generation Mesenteric Arterial Rings of WKY Rats and SHRs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>WKY (n=12/drug)</th>
<th>SHR (n=12/drug)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emax (%) pED50</td>
<td>Emax (%) pED50</td>
</tr>
<tr>
<td>Fenoldopam</td>
<td>81±8 5.0±0.2</td>
<td>46±10* 4.4±0.2*</td>
</tr>
<tr>
<td>PD128907</td>
<td>80±6 5.0±0.1</td>
<td>66±6* 4.5±0.9</td>
</tr>
<tr>
<td>Fenoldopam+PD128907</td>
<td>91±6† 5.6±0.2†</td>
<td>67±10* 3.9±0.01*</td>
</tr>
</tbody>
</table>

*P<0.01 vs WKY, †P<0.01 vs others; Emax=maximum relaxation induced by acetylcholine.
and sodium-phosphate cotransport activity in opossum kidney cells.1,5 The D₁ and D₃-like receptors that interact in renal tubule cells are not known. There are at least two D₁-like receptors, the D₁ and the D₃ receptor, expressed in the kidney. The D₁ receptor is responsible for most of the increase in cAMP production in renal proximal tubule cells after stimulation with D₁-like agonist, fenoldopam. Therefore, we have suggested that the D₁ receptor rather than the D₃ receptor is responsible for the natriuretic effect of D₁-like agonists.8 The current studies show that the D₁ and the D₃ receptor interact with each other in RPT cells from normotensive rats. Whether the ability of D₁ receptors to stimulate D₃ receptor expression and increase their interaction plays a role in the natriuretic effect of dopamine remains to be determined. We do show that acute (30 minutes) and long-term interactions (24 hours) are impaired in RPT cells from SHRs. An impaired interaction between D₁-like and D₃-like receptors in renal proximal tubules has been shown in SHRs, but the dopamine receptor subtypes involved were not determined.27 A preferential D₁ receptor agonist and two selective D₁ receptor agonists, R(++)-7-hydroxypropyl-aminotetralin and PD128907, increase sodium excretion in normotensive but not in hypertensive rats.18,28,29 Chronic blockade of D₃ receptors induces hypertension in Dahl salt-resistant rats;28 mice lacking the D₃ receptor are hypertensive and have a decreased ability to excrete an acute saline load.30 Several laboratories, including ours, have also shown that renal D₁ receptor function is also impaired in genetic hypertension.1,2 It is not known, however, whether the dopaminergic defect in genetic hypertension is primarily caused by the D₁ or D₃ receptor.

Our studies show that in the maximally vasorelaxed mesenteric artery, a D₁ receptor agonist (fenoldopam) or a D₁ receptor agonist, PD128907, a 120-fold selectivity over the D₃ receptor26 has no effect on vascular contractility, in agreement with our previous report.23 The current studies also support our previous report of the vasorelaxation effect of D₁ or D₃ receptors; D₁-like receptor-induced vasorelaxation is increased with D₃ receptor activation in WKY rats.23 The vasorelaxant effects of fenoldopam and PD128907 in mesenteric vessels are attenuated in SHRs, although the impairment is greater with fenoldopam than with PD128907. Moreover, in SHRs, unlike the response seen in WKY rats, D₁ receptor stimulation does not enhance the vasodilatory effect of D₃ receptor stimulation. Although fenoldopam is a potent vasodilator of several vascular beds in SHRs and humans with essential hypertension,31–33 a blunted renal vasodilatory effect has also been reported in SHRs and humans with essential hypertension.34–36

We have previously reported that D₁ and D₃ dopamine receptor subtypes physically interact with and regulate each other’s expression in A10 aortic vascular smooth muscle cells.23 The effect of D₁-like receptors on D₁ and D₃ receptor expressions and their interaction was not studied in mesenteric arterial vascular smooth muscle cells because such cells are not available. We chose to study instead, arterial vascular smooth muscle cells from human coronary arteries.16 The results are similar to those found in RPT cells and A10 cells from normotensive rats.23 However, the ability of the D₁ and D₃ receptors to regulate each other’s expression in RPT cells from normotensive rats and CASMCSs from humans occur only after 2 hours of agonist incubation. Therefore, other mechanisms are probably involved in the enhancement of the vasodilatory effect of D₁ receptors by D₃ receptors, eg, increase in signaling molecules.

The D₁ and D₃ receptors heterologously expressed in Chinese hamster ovary cells (D₁>D₃) act synergistically to increase arachidonic acid release.37 This is an unlikely mechanism for the positive interaction of D₁ and D₃ receptors because dopamine and D₃ receptor agonists actually decrease arachidonic acid release.38 It is also unlikely that nitric oxide or any endothelium-derived relaxing factor is involved because we have found that removal of endothelial cells does not impair the vasorelaxant effect of D₁ and D₃ receptors.23 We have suggested that the D₁ receptor-mediated vasorelaxation in vascular smooth muscle cells23 is caused by stimulation of K⁺ channels.40 Under these conditions, the vasorelaxant effects of D₁-like receptors caused by protein kinase A could be additive to the vasorelaxant effect of D₃ receptors caused by stimulation of K⁺ channels.

In summary, D₁ receptor increases D₁ and D₃ receptor protein expressions in cells from normotensive rat RPTs and human coronary arteries. In addition, D₁ and D₃ receptors relax mesenteric arterial rings and the vasorelaxation are increased by combined D₁ and D₃ receptor stimulation.

Perspectives

The impairment of dopamine receptor-induced vasodilation of mesenteric arterial vessels is greater for D₁ than D₃ receptors in SHRs. It is therefore possible that the dopaminergic defect in genetic hypertension is primarily related to D₁ receptor dysfunction. The cause of the impaired D₁-like receptors in RPT cells of SHRs is caused by an uncoupling of the D₁-like receptor by increased activity of G protein-coupled receptor kinases and decreased activity of protein phosphatase 2A.1 The cause of the impaired D₁ receptor function in vascular smooth cells in SHRs remains to be determined. Whether vascular smooth muscle cells from resistance vessels in SHRs respond to D₁-like receptor stimulation similarly to RPT cells from SHRs remains to be determined.

Acknowledgments

These studies were supported in part by grants from the National Institutes of Health, HL 23081, DK 39308, DK52612, HL 62211, and HL 41618.

References


Aberrant D₁ and D₃ Dopamine Receptor Transregulation in Hypertension
Chunyu Zeng, Dan Wang, Laureano D. Asico, William J. Welch, Christopher S. Wilcox, Ulrich Hopfer, Gilbert M. Eisner, Robin A. Felder and Pedro A. Jose

Hypertension. 2004;43:654-660; originally published online January 19, 2004;
doi: 10.1161/01.HYP.0000114601.30306 bf
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/43/3/654

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/