Dopamine D<sub>1</sub>-Like Receptors Suppress the Proliferation of Macrophages Induced by Ox-LDL

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Kew Words
Dopamine D<sub>1</sub>-like receptors • Oxidized low-density lipoprotein (Ox-LDL) • Macrophage • Proliferation

Abstract

**Background/Aims:** Oxidized low-density lipoprotein (Ox-LDL) induces macrophage proliferation, a key physiological process which leads to atherosclerosis. The aim of this study was to determine the effects of dopamine D<sub>1</sub>-like receptors on macrophage proliferation induced by Ox-LDL. **Methods:** The expression of dopamine D<sub>1</sub>-like receptors was determined by immunohistochemistry, reverse transcriptase-polymerase chain reaction (RT-PCR) and immunoblotting. The effect of D<sub>1</sub>-like receptors on macrophage proliferation induced by Ox-LDL was measured by [3H]-thymidine incorporation and cell number count. **Results:** Dopamine D<sub>1</sub>-like receptors were present in macrophages as determined by immunohistochemistry, RT-PCR and immunoblotting. A D<sub>1</sub>-like receptor agonist, fenoldopam, which by itself had no effect on macrophage proliferation, inhibited the stimulatory effect of Ox-LDL on macrophage proliferation. This was further confirmed by the D<sub>1</sub>-like receptor antagonist SCH 23390 blocking the effect of fenoldopam, thereby indicating that the fenoldopam action was receptor specific. Phosphatidylinositol 3-kinase (PI3K/Akt) and mitogen-activated protein kinase (MAPK/ERK) pathways were also involved in the proliferative effect of Ox-LDL because in the presence of PI3K/Akt or MAPK/ERK inhibitors, LY294002 or PD98059, the stimulatory effects of Ox-LDL were blocked. Moreover, the stimulatory effect of Ox-LDL on the phosphorylation of ERK and Akt was significantly reduced by fenoldopam in macrophages. Additional experiments found that both D<sub>1</sub> and D<sub>5</sub> receptor expression was lower in the peritoneal macrophages from Apolipoprotein E-deficient mice compared to the control C57Bl/6J mice. **Conclusions:** Macrophages express D<sub>1</sub>-like receptors. The activation of the D<sub>1</sub>-like receptors significantly inhibits Ox-LDL-induced macrophage proliferation, possibly through the inhibition of the PI3K/Akt and MAPK/ERK signaling pathways.

Y. Yao and D. Yang contributed equally to this work.
Introduction

Atherosclerosis is a major pathological mechanism of cardiovascular diseases. One of the characteristic events in the formation of atherosclerotic lesions is the proliferation of cells, including vascular smooth muscle cells and macrophages, within the arterial walls [1, 2]. Macrophage-derived foam cells are believed to play an important role in the development and progression of atherosclerosis through the production of various bioactive molecules [3]. Oxidized low-density lipoprotein (Ox-LDL), a major risk factor, is involved in the pathogenesis of atherosclerosis, ascribed in part to the Ox-LDL-induced macrophage proliferation [4-6]. Among the proliferative signaling pathways of Ox-LDL, phosphatidylinositol 3-kinase (PI3K/Akt) and mitogen-activated protein kinase (MAPK/ERK) are considered to be important.

Dopamine is an endogenous catecholamine that regulates many cellular functions, including behavior, hormone synthesis and release, blood pressure, and transmembrane ion transport [7]. Dopamine receptors are classified into the D1-like and D2-like subtypes based on their structure and pharmacology. D1-like receptors, composed of the D1 and D5 receptors, stimulate adenylyl cyclase activity, whereas D2-like receptors, composed of the D2, D3, and D4 receptors, inhibit adenylyl cyclase activity and regulate/modulate the activity of several ion channels [8]. Both D1-like and D2-like receptors are widely distributed in the peripheral arteries, including the aorta and mesenteric artery [9-11].

Studies from our laboratory [12] and others [13-15] have shown that activation of the dopamine D1-like receptors prevents atherosclerosis by inhibiting proliferation, migration and antioxidation of vascular smooth muscle cells (VSMCs). However, whether there are dopamine D1-like receptors in macrophages and their role in Ox-LDL-induced macrophage proliferation are unknown. Our present study was designed to examine the expression of dopamine D1-like receptors in RAW264.7 cells, a macrophage cell line, and investigate the effect of the D1-like receptors on RAW264.7 cell proliferation induced by Ox-LDL. We demonstrated that macrophages express dopamine D1-like receptors, and the activation of D1-like receptors significantly inhibited Ox-LDL-induced macrophage proliferation, possibly through the inhibition of the PI3K/Akt and MAPK/ERK pathways. Furthermore, the expression of dopamine D1-like receptors was lower in the peritoneal macrophages from Apo lipoprotein E-deficient mice compared to the control C57Bl/6j mice, indicating that dopamine D1-like receptors might be involved in atherosclerosis.

Materials and Methods

Cell culture and sample preparation

Peritoneal macrophages were collected from anesthetized male C57Bl/6j or apoE-/- mice (25–30 g) and identified with the adherent culture method as previously described [6, 16-18]. Briefly, 6-8 ml of pre-cooled RPMI-1640 medium (Gibco, Grand Island, NY) was injected into the mouse abdominal cavity. The mouse abdomen was gently kneaded for approximately 3 min, and the injected media was then aspirated out, placed into conical tubes and centrifuged at 1000 g for 15 min. The aspirate was then suspended in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Gibco) and cultured at 37°C in a 95% air/5% CO2 atmosphere. After approximately 2 h, the non-adherent cells were removed by gently washing three times with pre-warmed RPMI-1640 medium. More than 98% of the adherent cells were confirmed to be macrophages.

RAW264.7 cells (ATCC, Hercules, CA) were cultured at 37°C in a 95% air/5% CO2 atmosphere in RPMI-1640 medium to an estimated density of 2 × 10^6 to 5 × 10^6 cells/ml as previously described [18]. The human monocyte-like cell line, THP-1, was also obtained from ATCC and cultured in RPMI-1640 with 10% fetal bovine serum.

Peritoneal macrophages and RAW264.7 cells (80% confluence) were homogenized in ice-cold lysis buffer (pH 7.4) (20 mM Tris-HCl, pH 7.4; 2 mM EDTA, pH 8.0; 2 mM EGTA; 100 mM NaCl; 10 µg/ml leupeptin; 10 µg/ml aprotonin; 2 mM phenylmethylsulfonyl fluoride; 1% NP-40), sonicated, kept on ice for 1 h, and centrifuged at 16,000 g for 30 min. Protein concentration of the collected supernatant was determined.
using a protein assay kit (Bio-Rad Laboratories, Hercules, California) with bovine serum albumin used as the standard. The supernatants were boiled in sample buffer (35 mM Tris-HCl, pH 6.8, 4% SDS, 9.3% dithiothreitol, 0.01% bromophenol blue, 30% glycerol) at 95°C for 5 min. The samples were stored at -20°C until use.

**Immunoblotting**

Immunoblotting was performed as previously described [13, 19, 20]. Lysates from macrophages were prepared as previously described. Samples containing 50 μg of cell proteins were separated by SDS-PAGE in a 9% polyacrylamide gel and then electrotransferred to polyvinylidene difluoride membranes. The membrane was blocked in TBS (Tris-buffered saline) containing 5% non-fat dry milk for 2 h, and then incubated with primary antibodies [goat anti-mouse D$_1$R (1:200), rabbit anti-mouse D$_2$R (1:500), rabbit anti-mouse α-actin (1:400) (Santa Cruz, Dallas, TX) and rabbit anti-mouse Akt/ERK/Pi3K (1:800), rabbit anti-mouse phospho-Akt/ERK/Pi3K (1:600) (Cell Signaling, Boston, MA)] overnight at 4°C. The membranes were then further incubated with IRDye 800 infrared-labeled donkey anti-rabbit/monkey anti-goat secondary antibodies (Li-Cor Biosciences, Lincoln, NE) at room temperature for 1 h. The membranes were then washed three times with phosphate buffered saline (PBS)-Tween-20. The bound complex was detected using the Odyssey Infrared Imaging System (Li-Cor Biosciences). The images were analyzed using the Odyssey Application Software to obtain the integrated intensities.

**RT-PCR of D$_n$-like receptors**

A total of 2–3 μg of total RNA extracted from RAW264.7 cells was used to synthesize cDNA, which served as a template for the amplification of dopamine D$_n$-like receptors [12]. For the mouse dopamine D$_1$ receptor, the forward primer was: 5’-GTA GCC ATT ATG ATC GTCAC-3’ and the reverse primer was: 5’-GAT CAC AGA CAG TGT CTT CAG-3’; for the mouse dopamine D$_2$ receptor, the forward primer was: 5’-CTA CGA GCG CAA GAT GACC-3’ and the reverse primer was: 5’-CTC TGA GCA TGC TCA GCTG-3’; for the mouse β-actin, the forward primer was: 5’-CCT CTA TGC CAA CAC AGTGC-3’ and the reverse primer was: 5’-GGT CTC CTA TG CAC AGTGC-3’ and the reverse primer was: 5’-GTA CTC CTA TG CAC AGTGC-3’. The amplification was performed with the following conditions: denaturation at 94°C for 30 s, annealing for 30 s at 60°C, and extension for 45 s at 72°C for 35 cycles. PCR products were visualized in ethidium bromide-stained 2% agarose gels, and band sizes were compared with a 600 bp DNA ladder (Gibco).

**Immunohistochemistry**

Cells grown in 6-well plates were fixed for 30 min in PBS containing 4% paraformaldehyde and washed three times with PBS alone. Fixed cells were incubated with anti-dopamine D$_1$ and D$_2$ receptor antibody (1:200) at 4°C overnight. After incubation with the primary antibodies, cells were rinsed three times with PBS and incubated for 60 min at 37°C with 10 μg/ml of biotin-conjugated goat anti-rabbit or monkey anti-goat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). The cells were again washed three times with PBS and incubated for 20 min at room temperature with an avidin-biotin peroxidase complex (Elite ABC kit; Vector Laboratories Inc., Burlingame, California) followed by development for 10 min using 3-amino-9-ethylcarbazole and peroxide with a kit from Calbiochem. Images were obtained using a Leica DMLS microscope (Leica Microsystems, Buffalo Grove, IL) [21].

**[³H]-thymidine incorporation**

Cell proliferation was determined by measuring the incorporation of [³H]-thymidine (Atomic Energy Research Establishment of China, Beijing City, China) into the DNA of cells cultured in 96-well plates [22]. Human LDL was oxidized using Cu$_2$SO$_4$ (oxidant) in PBS. Oxidation was terminated by adding excess EDTA-Na$_2$. After induction of quiescence, the cells were stimulated with Ox-LDL (Yiyuan Co., Guangzhou City, China) or vehicle (dH$_2$O) in the presence or absence of fenoldopam, PD98059 or LY294002 (Sigma-Aldrich, St. Louis, MO) for 48 h. The antagonists and inhibitors were added to the medium 30 min prior to the corresponding agonists. Thereafter, [³H]-thymidine (1 mCi/ml) was added to the growth medium of each well 6 h prior to the measurements. At the end of the incubation period, the medium was removed and the cells were treated with 0.25 ml of 0.05% trypsin/0.53 mM EDTA (Gibco) for 5 min and diluted to 10 ml with a balanced electrolyte solution [12]. The cells were then treated with 10% trichloroacetic acid to precipitate acid-insoluble materials from which the DNA was extracted using 0.1 N 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 being counted in a liquid scintillation counter (Beckman LS6500, Beckman, MO). Protein concentrations were determined using a BCA protein assay kit (Pierce, Rockford, IL), as previously described [23]. Data are presented as $[^{3}H]$-thymidine uptake per microgram of protein.

To determine the specificity of the D$_1$-like receptors agonist, the D$_1$-like receptors antagonist SCH23390 (Sigma, St. Louis, MO) [11] was used to block the effect of fenoldopam.

**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)-2, 5-diphenyltetrazolium bromide (MTT). MTT is selectively taken up by mitochondria and 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 a density of 1×10$^5$ cells/well, the cells were incubated with the indicated reagents for 48 h. Subsequently, 20 μl of MTT (2.5 g/L) was added to each well, and the incubation continued for an additional 4 h at 37°C. Thereafter, 150 μl DMSO was added to each well, and absorbance was read at 490 nm on a microplate reader (model 680, Bio-Rad, Hercules, CA).

**Statistical analysis**

The data are expressed as the 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 was made by factorial ANOVA with a Holm-Sidak ad hoc test (or t-test when only 2 groups were compared). A value of P<0.05 was considered significant.

**Results**

The Ox-LDL-mediated proliferation of RAW264.7 cells is attenuated by the activation of dopamine D$_1$-like receptors

First, we assessed whether the D$_1$-like receptors, including the D$_1$ and D$_5$ receptors, were present in macrophages. It was shown that both the D$_1$ and D$_5$ receptors were present in macrophages, as determined by immunohistochemistry, immunoblotting and RT-PCR, using human umbilical vein endothelial cells (HUVEC, ATCC, Manassas, VA) as a negative control [24] and the embryonic thoracic aortic smooth muscle cells from normotensive Berlin-Druckrey IX rats (A10, ATCC, Manassas, VA) as a positive control [12, 13] (Fig. 1A-1C).

Consistent with other reports [1, 16, 25], Ox-LDL induced proliferation of RAW264.7 cells in a concentration-dependent manner (10-40 μg/ml), as determined by $[^{3}H]$-thymidine incorporation (Fig. 2A). Although fenoldopam alone had no effect on cell proliferation (Fig. 2B), it reduced the Ox-LDL-mediated RAW264.7 proliferation in a concentration (10$^{-9}$-10$^{-6}$ M)-dependent manner (Fig. 2C). The inhibitory effect of fenoldopam was determined to be via the D$_1$-like receptors because addition of a D$_1$-like receptor antagonist, SCH23390 (10$^{-7}$ M), blocked the inhibitory effect of fenoldopam (10$^{-7}$ M) (Fig. 2D). The $[^{3}H]$-thymidine incorporation was also confirmed by cell count and MTT, indicating that fenoldopam, via D$_1$-like receptors, reduced the Ox-LDL-mediated macrophage cell proliferation (Fig. 2E, 2F and 2G). To further confirm this result, we used another human macrophage line (THP-1). The results indicated that fenoldopam inhibited Ox-LDL-induced cell proliferation in the presence of a D$_1$R antagonist (SCH23390 or SKF83566), as the inhibitory effect of fenoldopam was blocked (Fig. 2G).

**Role of PI3K/Akt and MAPK/ERK signaling pathways in the inhibitory effect of fenoldopam on Ox-LDL-induced proliferation of RAW264.7 cells**

PI3K/Akt and MAPK/ERK are known to be important signaling molecules in cell proliferation, migration and apoptosis [1, 12, 16, 26]. Consistent with previous studies [4, 6, 27, 28], our results showed that the Ox-LDL-mediated proliferation was via the PI3K/Akt and MAPK/ERK pathways because in the presence of PI3K/Akt or MAPK/ERK inhibitors, PD98059 (10$^{-7}$ M) and LY294002 (10$^{-5}$ M), respectively, the Ox-LDL (20 μg/ml)-mediated
Yao et al.: Effect of Dopamine Receptor on Macrophages Proliferation

The proliferation of RAW264.7 cells was inhibited (Fig. 3A). Further analysis showed that Ox-LDL increased phosphorylation of ERK and Akt, while addition of fenoldopam reduced the Ox-LDL-mediated phosphorylation of ERK or Akt (Fig. 3B-3F). We further investigated the underlying mechanisms of \( \text{D}_1 \)-like receptors on the inhibition of Ox-LDL-induced p-ERK. Our results showed that the effect of fenoldopam was through the cAMP/PKA pathway because in the presence of a PKA inhibitor, 14 - 22 amide, the inhibitory effect of fenoldopam on Ox-LDL-induced p-ERK was blocked; while addition of forskolin, an adenylyl cyclase activator, augmented the effect of fenoldopam on Ox-LDL-induced p-ERK (Fig. 3G).

The expressions of \( \text{D}_1 \)-like receptors are decreased in peritoneal macrophages obtained from \( \text{apoE}^{-/} \) mice

The Apolipoprotein E-deficient mouse is an animal model of atherosclerosis with high levels of Ox-LDL [2]. Due to the inhibitory effect of fenoldopam on macrophage proliferation, we investigated whether the dopamine receptor expression is aberrant in \( \text{apoE}^{-/} \) mouse cells. We found that both the \( \text{D}_1 \) and \( \text{D}_5 \) receptor expression in peritoneal macrophages was lower compared to the control mice (Fig. 4A). We observed that treatment with Ox-LDL led to the decreased expression of \( \text{D}_1 \) and \( \text{D}_5 \) receptors in vitro, suggesting that hyperlipidemia may also play a major role in the inhibition of the receptor expression (Fig. 4B).

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**Fig. 1.** Expression of dopamine \( \text{D}_1 \)-like receptors in mouse macrophages. Dopamine \( \text{D}_1 \) and \( \text{D}_5 \) receptor expression in RAW264.7 was determined by immunohistochemistry (A), RT-PCR (B) and immunoblotting (C), using HUVEC as the negative control and embryonic thoracic aortic smooth muscle cells from normotensive Berlin-Druckrey IX rats (A10) as the positive control. In B, lane 1: HUVEC; lane 2: A10 cells; lane 3: RAW264.7 cells.
Fig. 2. Effect of D₁-like receptors on Ox-LDL-induced proliferation of RAW264.7 cells. (A) Concentration-dependent stimulatory effect of Ox-LDL on RAW264.7 cell proliferation. The proliferation of RAW264.7 cells was determined by [³H]-thymidine incorporation after incubation with the indicated concentrations of Ox-LDL (10-40 µg/ml) for 48 h. The control is vehicle (dH₂O). The results are expressed as counts per minute (cpm)/mg protein (n = 6, *P < 0.05 vs. control). (B and C) Effect of fenoldopam on RAW264.7 cell proliferation. RAW264.7 cells were treated with different concentrations of fenoldopam (10⁻⁹–10⁻⁷ M) (B) with or without Ox-LDL (20 µg/ml/48 h) (C). The proliferation of RAW264.7 cells was determined by [³H]-thymidine incorporation. The results are expressed as counts per minute (cpm)/mg protein (n = 5-6, *P < 0.05 vs. control).
The inhibitory effect of fenoldopam is via the cAMP/PKA pathway because the effect
Moreover, we also found that fenoldopam reduced the stimulatory effect of Ox-LDL on the
macrophages may be through inhibition of PI3K/Akt and MAPK/ERK signaling pathways.

Discussion

Previous studies have shown that the proliferation of cells, including vascular smooth
muscle cells and macrophages, within arterial walls is a major event in the formation of
atherosclerotic lesions [4, 6, 12, 24, 29]. In vitro and in vivo studies strongly suggest that
macrophages and macrophage-derived foam cells proliferate in atherosclerotic lesions and
may be involved in the enhanced progression of atherosclerosis in vivo [6, 30-32]. Ox-LDL
is an important risk factor, and although the mechanisms are complex, it is believed that
the stimulatory effect of Ox-LDL on macrophage proliferation plays an important role [1,
6, 24]. Dopamine receptors are expressed in many organs and tissues, and stimulation of
the dopamine D$_1$-like receptors prevents atherosclerosis via the inhibition of proliferation,
migration and oxidation of VSMCs [12-15]. Dopamine D$_1$-like receptors are present in
VSMCs, platelets, lymphocytes and natural killer cells [33, 34]. Our current study shows that
both D$_1$ and D$_3$ receptors are expressed in macrophages. Activation of the D$_1$-like receptors
reduced the Ox-LDL-induced proliferation in both RAW264.7 and THP-1 cells, indicating that
they are involved in the pathogenesis of atherosclerosis as D$_1$ and D$_3$ receptor expression in
peritoneal macrophages are lower in apoE$^-$ compared to the C57Bl/6J control mice.

The mechanisms of Ox-LDL-induced macrophage proliferation are complex and include
processes such as an increase in intracellular calcium, activation of protein kinase C (PKC),
and secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) [35-37].
PI3K/Akt and MAPK/ERK are believed to be involved in downstream signaling [4, 26, 38].
The MAPK signaling pathway includes ERK, JNK and p38 MAPK; the ERK pathway is the
most common cell proliferative response-mediated signaling pathway [1, 39]. PI3K/Akt
is one of the major signaling pathways in cell proliferation and has been identified as an
important factor in cancer [40]. When PI3K is activated, it generates a second messenger in
the cell membrane: PIP$_3$, which binds to and activates Akt. Akt, also known as protein kinase
B (PKB), has a relatively large molecular mass of 60,000 Da and phosphorylates a variety
of transcription factors directly and can therefore inhibit apoptosis gene expression and
enhance the anti-apoptotic gene expression, thereby promoting cell survival [1, 26]. In the
present study, we first confirmed that the Ox-LDL-mediated proliferation was via the PI3K/
Akt and MAPK/ERK pathways. Then, we found that stimulation with fenoldopam, the D$_1$-
like receptor agonist, decreases the Ox-LDL-induced ERK and Akt phosphorylation, which
suggests that the inhibitory effect of D$_1$-like receptors on Ox-LDL-induced proliferation of
macrophages may be through inhibition of PI3K/Akt and MAPK/ERK signaling pathways.
Moreover, we also found that fenoldopam reduced the stimulatory effect of Ox-LDL on the
p-ERK. The inhibitory effect of fenoldopam is via the cAMP/PKA pathway because the effect
Fig. 3. Role of MAPK and PI3K on the anti-proliferative effects of D₁-like receptors in RAW264.7 cells. (A) Effect of MAPK and PI3K on the Ox-LDL-mediated proliferation of RAW264.7 cells. RAW264.7 cells were treated with MAPK/ERK and PI3K/Akt inhibitors, PD98059 (10⁻⁷ M) or LY294002 (LY) (10⁻⁵ M), respectively, with or without Ox-LDL (20 µg/ml/48 h). The proliferation of RAW264.7 cells was determined by [³²]H-thymidine incorporation. The results are expressed as cpm/mg protein (n = 6, * P < 0.05 vs. control; # P < 0.05 vs. Ox-LDL). (B and C) Effect of fenoldopam on phosphorylated ERK expression in RAW264.7 cells. Cells were stimulated with Ox-LDL (20 µg/ml) (B) and/or fenoldopam (10⁻⁹-10⁻⁷ M) (C) for different times (15 min/5-60 min). The expressions of p-ERK and total ERK were determined by immunoblotting (n = 4, * P < 0.05 vs. control; # P < 0.05 vs. Ox-LDL). (D and E) Effect of fenoldopam on phosphorylated Akt expression in
RAW264.7 cells. Cells were stimulated with Ox-LDL (20 µg/ml) (D) and/or fenoldopam (10^{-9}-10^{-7} M) (E) for different times (15 min/5-60 min). The expression of p-Akt and total-Akt was determined by immunoblotting (n = 4, * P < 0.05 vs. control; # P < 0.05 vs. Ox-LDL). (F) Effect of fenoldopam on phosphorylated PI3K expression in RAW264.7 cells. Cells were stimulated with Ox-LDL (20 µg/ml) (D) and/or fenoldopam (10^{-9}-10^{-7} M) for different times (15 min/5-60 min). The expressions of p-PI3K and total PI3K were determined by immunoblotting (n = 4, * P < 0.05 vs. control; # P < 0.05 vs. Ox-LDL). (G) The role of the cAMP/PKA pathway in the inhibitory effect of fenoldopam on Ox-LDL-induced p-ERK. Cells were incubated with the indicated reagents (forskolin, 10^{-5} M; PKA inhibitor, 14-22 amide [PKAI], 10^{-6} M; fenoldopam [Fen], 10^{-7} M, and Ox-LDL, 20 µg/ml) for 15 min. The expressions of p-ERK and total-ERK were determined by immunoblotting (n = 7, * P < 0.05 vs. control; # P < 0.05 vs. Ox-LDL).

**Fig. 4.** The expression of D_{1}-like receptors in peritoneal macrophages obtained from apoE^{-/-} mice. (A) The peritoneal macrophages obtained from anesthetized C57Bl/6J or apoE^{-/-} mice (weight 25–30 g). D_{1} or D_{5} receptor expressions were determined by immunoblotting (n = 3, * P < 0.05 vs. C57Bl/6J mice). (B) Effect of Ox-LDL on D_{1} or D_{5} receptor expressions in RAW264.7 cells. RAW264.7 cells were treated with Ox-LDL (20 µg/ml/48 h). D_{1} or D_{5} receptor expressions were determined by immunoblotting (n = 6, * P < 0.05 vs. control).

was enhanced by the adenylyl cyclase activator, forskolin, but blocked by the PKA inhibitor, 14-22 amide. In fact, our results and work by others have also found that activation of the D_{1}-like receptors in VSMCs suppresses the activated MAPK and PI3k/mTOR pathways as well as the PLD and PKC activities [14, 15, 41].

Our own publications and studies by others have shown the physiological functions of the dopamine receptors on vascular diseases, including anti-atherosclerotic activities [12, 14, 15, 41]. Activation of the D_{1}-like receptors inhibits VSMC proliferation and migration induced by factors such as insulin, platelet-derived growth factor (PDGF) BB, and insulin-like growth factor-1 (IGF-1) [12, 14, 15, 41]. D_{1}-like receptors also suppress the insulin- and angiotensin II-induced VSMC proliferation and migration [42-44]. Moreover, stimulation
of both dopamine D$_1$-like and D$_2$-like receptors leads to vasorelaxation independent of the endothelium, which is impaired in obese Zucker rats [45, 46]. In the present study, we further found that activation of the D$_1$-like receptors reduces the Ox-LDL-induced proliferation in macrophages, which extends the physiological functions of the D$_1$-like receptors on the artery. Because D$_1$-like and D$_2$-like receptors synergistically increase sodium excretion and induce vasorelaxation [45, 47], it is possible that the D$_1$-like receptors, together with the D$_2$-like receptors, synergistically inhibit the proliferation of macrophages. However, this hypothesis needs to be confirmed in future studies.

In conclusion, we have demonstrated that D$_1$-like receptors are present in mouse macrophages, and the proliferative effect of Ox-LDL in macrophages is significantly reduced by the activation of the D$_1$-like receptors, possibly through the PI3K/Akt and MAPK/ERK signaling pathways.

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Disclosure Statement

No conflicts of interest, financial or otherwise, are declared by the authors.

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Yao et al.: Effect of Dopamine Receptor on Macrophages Proliferation

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