

# Platelet-Derived Growth Factor B Chain Is a Novel Target Gene of Cocaine-Mediated Notch1 Signaling: Implications for HIV-Associated Neurological Disorders

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Neuroinflammation associated with HIV-1 infection is exacerbated in cocaine-abusing, HIV+ individuals. The underlying mechanisms are, in part, attributable to disruption of the blood–brain barrier modulated by cocaine via platelet-derived growth factor B chain (PDGF-B). Since Notch signaling plays a critical role in CNS homeostasis, we hypothesized that it may have a role in cocaine-mediated induction of PDGF-B. The goal of this study was to link Notch signaling with PDGF-B. Using Western blot analysis, we demonstrate the role of Notch1 signaling in cocaine-mediated induction of PDGF-B in human brain microvascular endothelial cells. Exposure of cells to the  $\gamma$ -secretase inhibitor-DAPT or silencing of Notch1 resulted in abrogation of cocaine-mediated induction of PDGF-B. Reciprocally, activation of the Notch1 receptor by exposing cells to the Notch ligand Jagged-1 resulted in upregulation of PDGF-B expression. Furthermore, it was demonstrated that cocaine-mediated activation of Notch1 signaling leading to targeted expression of PDGF-B involved activation of the downstream effector CSL. Functional implication of Notch1 signaling in regulating expression of the vascular permeant PDGF-B was confirmed *in vitro* using cell permeability assays. *In vivo* relevance was further corroborated in cocaine-treated mice that demonstrated increased permeability of the endothelial barrier as evidenced by Evans blue and sodium fluorescein extravasation. Specificity of Notch1 signaling *in vivo* was validated in mice exposed to DAPT, which failed to demonstrate barrier disruption following cocaine exposure. This is the first evidence of involvement of Notch1 activation in cocaine-mediated regulation of PDGF-B expression.

## Introduction

Although the advent of combined anti-retroviral therapy has significantly decreased the incidence of HIV-associated neurological disorders (HAND), prevalence of the disease is actually on the rise (Sacktor et al., 2002; Antinori et al., 2007). Drug abuse has been implicated as a contributing risk factor for increased blood–brain barrier (BBB) disruption and the ensuing neuroinflammation associated with HIV-1 infection (Webber et al., 1999; Nair et al., 2004). BBB is critical for the maintenance of CNS homeostasis and for the regulation of neuronal microenvironment. Intriguingly, cocaine use has been shown to open up the BBB (Fiala et al., 1998, 2005; Zhang et al., 1998). The mechanisms underlying the ability of cocaine to induce BBB damage however, remain elusive.

Previous reports have indicated that PDGF signaling is critical for vascular development and for blood vessel homeostasis (Jin et al., 2008). More recently, intriguing findings by Su et al. (2008) have identified PDGF released from astrocytes as a key mediator of disruption of cerebrovascular permeability leading to increased risk for stroke. Consistent with this finding, we have also recently demonstrated PDGF-B as a regulator of cocaine-mediated BBB damage both *in vitro* and *in vivo* (Yao et al., 2011a), thereby underpinning the role of this factor in orchestrating BBB permeability.

Notch signaling has emerged as an important regulator of neuronal homeostasis. Similar to the physiological effects of PDGF, Notch signaling also mediates intercellular signals that affect proliferation, survival, and differentiation of endothelial cells (MacKenzie et al., 2004; Nosedá et al., 2004). Notch receptors (Notch1 to 4) are transmembrane proteins, binding of ligands such as Delta and Jagged-1 resulting in proteolytic cleavage of the Notch receptors by the  $\gamma$ -secretase enzyme complex, leading to the release of a Notch intracellular domain (NICD). NICD subsequently translocates to the nucleus, where it interacts with the DNA binding factor CSL (also known as RBP-Jk and CBF1), ultimately culminating into transactivation of various promoters, such as those for the HES and HEY families. Additional immediate downstream genes of Notch signaling have also been characterized, suggesting the existence of a larger immediate Notch transcriptome. As an example, PDGF- $\beta$ R has been shown to be a target of Notch signaling gene in vascular smooth muscle

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cells (Jin et al., 2008). Whether Notch signaling is also involved in induction of the ligand PDGF-B, however, remains elusive. In this report, we demonstrate that Notch1 signaling is upstream of PDGF-B transcription in human brain microvascular endothelial cells (HBMECs) and identify it as a new member of the immediate Notch downstream target gene that plays a crucial role in BBB integrity.

## Materials and Methods

**Animals.** C57BL/6N mice were purchased from Charles River Laboratories. All the animals were housed under conditions of constant temperature and humidity on a 12 h light, 12 h dark cycle, with lights on at 7:00 A.M. Food and water were available *ad libitum*. Animals were deeply anesthetized by overdose of isoflurane followed by pneumothorax before perfusion. All animal procedures were performed according to the protocols approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center.

**Reagents.** Cocaine,  $\gamma$ -secretase inhibitor DAPT (*N*-[*N*-(3,5-difluorophenacetyl)-*L*-alanyl]-*S*-phenylglycine *t*-butyl ester), and anti-Flag antibody were purchased from Sigma-Aldrich. Jagged-1 was obtained from ANASPEC. Flag-tagged CSL-VP16 and LNCX retroviral vectors for the overexpression of CSL were obtained from Dr. Aly Karsan (University of British Columbia, Vancouver, BC, Canada).

**Cell culture.** Primary HBMECs obtained from Dr. Monique Stins (Johns Hopkins University, Baltimore, MD) were cultured as described previously (Yao et al., 2011b). HBMECs were pretreated with pharmacological inhibitor (DAPT: 10  $\mu$ M or 50  $\mu$ M) and Notch ligand (Jagged-1: 1  $\mu$ M or 10  $\mu$ M) for 1 h followed by cocaine (1, 10, or 100  $\mu$ M) exposure.

**Reverse transcription and real-time PCR.** Reverse transcription (RT) and real-time PCR assays were performed as described previously (Yao et al., 2009, 2010). The primers for human CSL (GenBank accession number NM005349) were as follows: upstream primer, 5'-CAGGCCACTCCATGTCCAA-3'; and downstream primer, 5'-ACCACAGGCACAGGAGTGACT-3'. The real-time PCR primer sequences for Hes1 and Hes5 were designed according to the previously published report (Kolev et al., 2008).

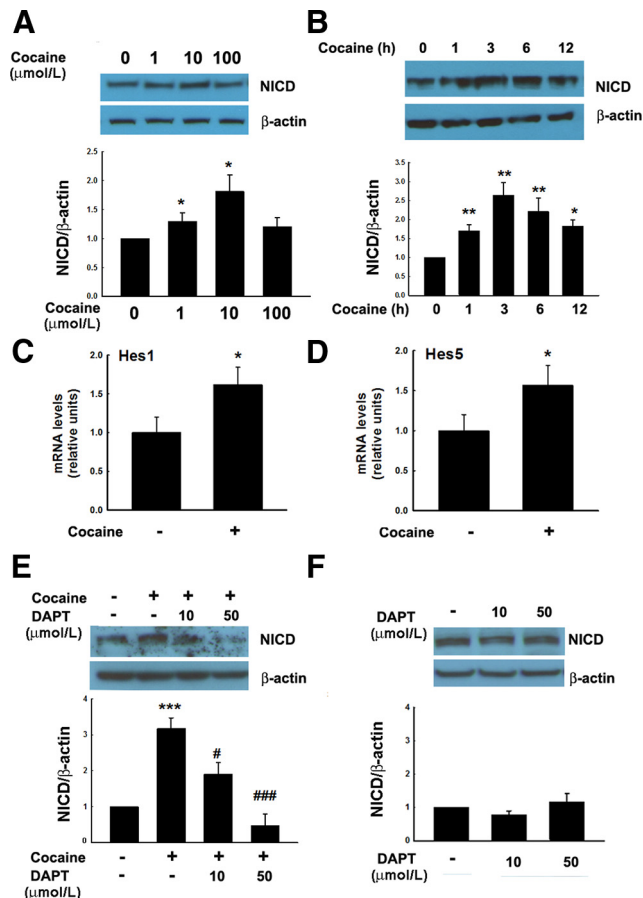
**Western blotting.** Treated cells were lysed using the Mammalian Cell Lysis kit (Sigma) as described earlier (Yao et al., 2009). The primary antibodies used were as follows: cleaved Notch1 (1:200, Cell Signaling Technology), PDGF-B (1:1000, Santa Cruz Biotechnology), and  $\beta$ -actin (1:4000, Sigma).

**Short interfering RNA transfection.** HBMEC cells were transfected with 20 nmol/L short interfering RNA (siRNA) for Notch1 and CSL from Dharmacon. The knockdown efficiency of siRNA Notch 1 and CSL was determined 2 d after transfection by Western blotting (WB) and RT-PCR analyses, respectively.

**Chromatin immunoprecipitation assay.** Chromatin immunoprecipitation assay was performed according to the manufacturer's instructions (Millipore). Briefly, HBMECs transfected with Flag-tagged CSL construct were treated with cocaine for 3 h and used for these assays as described previously (Yao et al., 2010, 2011a,b). Purified DNA was amplified via PCR to identify the promoter region containing the CSL binding site "TGGGAA." The sequence of the primers used to identify the PDGF-B promoter bound by CSL was as follows: sense: 5'-CTGTGTGAAGGCTGGTTGTG-3', antisense: 5'-CCGAGTCTCCCTCCTAGC-3'.

**Cell permeability.** Primary HBMECs seeded on 6.5 mm polyester Transwell inserts (0.4  $\mu$ m pore size) were grown to confluence. Following confluency monolayers were exposed to cocaine (10  $\mu$ M/L), Jagged-1 (1 or 10  $\mu$ M/L), DAPT (10  $\mu$ M/L), neutralizing PDGF-B antibody (500 ng/ml), or an isotype antibody, and the cell permeability was assessed as described earlier (Yao et al., 2011a).

**Assessment of the BBB integrity.** Assay of BBB integrity was performed in C57BL/6 mice. Mice were divided into four groups ( $n = 6$ ): (1) Saline, (2) Cocaine, (3) Cocaine plus DAPT, and (4) Cocaine plus DMSO. Cocaine was injected daily at a dose of 20 mg/kg (i.p.) for 7 d. For the DAPT study, mice were injected with cocaine for 7 d and additionally on days 1,



**Figure 1.** Cocaine-mediated increase of NICD levels in HBMECs. **A**, Effect of cocaine (1, 10, and 100  $\mu$ M) on NICD level in HBMECs. Whole-cell lysates from cocaine exposed cells (3 h) were subjected to WB using NICD antibody. **B**, Cocaine (10  $\mu$ M) induced time-dependent induction of NICD in HBMECs at the indicated time-points. **C**, **D**, HBMECs were treated with 10  $\mu$ M cocaine for 3 h and the isolated total RNA was assessed for Hes1 (**C**) and Hes5 (**D**) mRNA by real-time PCR analysis. **E**, DAPT is critical for cocaine-induced increase of NICD levels. Pretreatment of HBMECs with DAPT (10 or 50  $\mu$ M) for 1 h followed by cocaine exposure (3 h) significantly inhibited cocaine-induced increase of NICD levels. **F**, Immunoblots showing basal level NICD in HBMECs treated with DAPT (10 or 50  $\mu$ M) for 3 h. Representative immunoblots and the densitometric analyses of NICD/ $\beta$ -actin from four individual experiments are presented. All data are presented as mean  $\pm$  SD. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  versus control group; # $p < 0.05$ , ### $p < 0.001$  versus cocaine-treated group.

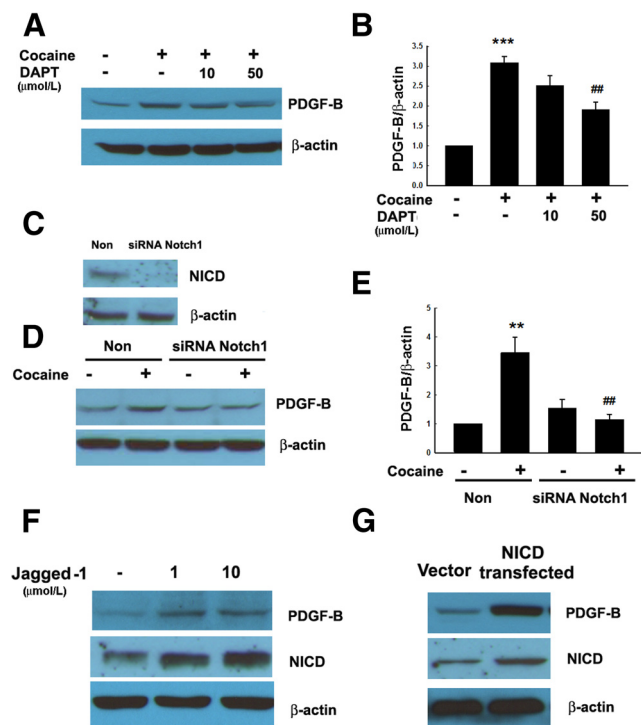
3, and 5 of cocaine injection, either DAPT or vehicle control DMSO (40 mg/kg, i.p.; same volume of DMSO) was also administered. The dose of DAPT chosen was based on previous report (Arumugam et al., 2006). One day after the last cocaine injection (eighth day), animals were injected in the tail vein with 200  $\mu$ l of sodium fluorescein (6 mg/ml; Sigma) and evaluated for BBB permeability as previously described (Chen et al., 2009; Yao et al., 2011a). To further validate BBB permeability, another tracer such as Evans blue dye (2%, 4 ml/kg) was administered through the tail vein as described previously (Arumugam et al., 2006; Daneman et al., 2010).

**Statistical analysis.** Statistical analysis was performed using one-way ANOVA with a *post hoc* Student's *t* test. Results were judged statistically significant if  $p < 0.05$  by ANOVA.

## Results

### Cocaine-mediated activation of NICD in HBMECs

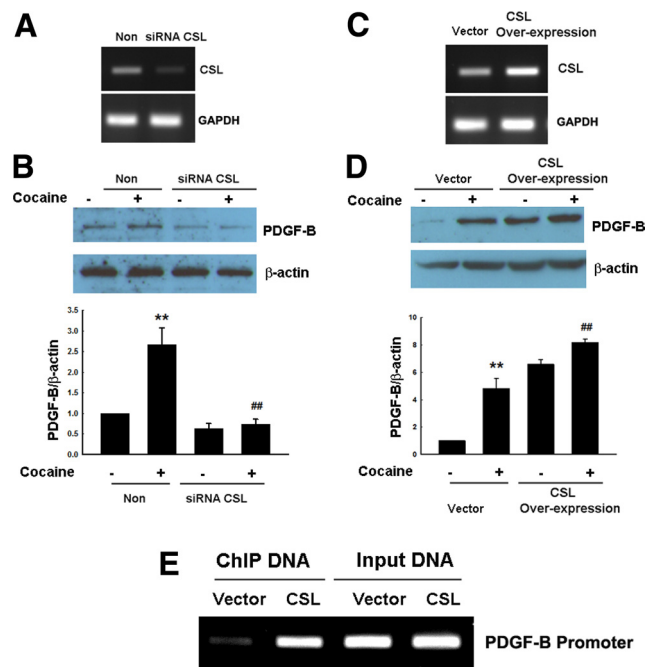
Since Notch is known to play a critical role in blood vessel disorders (MacKenzie et al., 2004; Nosedá et al., 2004) and cocaine is known to increase the permeability of blood vessels (Yao et al., 2011a), we reasoned that exposure of HBMECs to cocaine could involve activation of Notch1 signaling. Activation of Notch sig-



**Figure 2.** PDGF-B is a novel Notch1 immediate downstream target gene. **A, B,** Cocaine-mediated induction of PDGF-B was significantly attenuated in presence of DAPT (50  $\mu$ mol/L). **C, D,** Whole-cell lysates from HBMECs transfected with either Notch1/nonsense (Non) siRNAs were subject to WB. **D, E,** Notch1 siRNA but not Non siRNA inhibited cocaine-mediated induction of PDGF-B. HBMECs transfected with either Notch1 or Non siRNAs were treated with cocaine (10  $\mu$ mol/L) for 12 h and assessed for PDGF-B expression. **F,** Treatment of cells with Jagged-1 (1 or 10  $\mu$ mol/L) for 12 h induced PDGF-B expression. **G,** HBMECs were transfected with either an empty plasmid or a plasmid vector encoding NICD for 24 h and assessed for PDGF-B expression. Representative immunoblots and the densitometric analyses of PDGF-B/ $\beta$ -actin from four individual experiments are presented. All data are presented as mean  $\pm$  SD. \*\* $p$  < 0.01, \*\*\* $p$  < 0.001 versus control group; ## $p$  < 0.01 versus cocaine-treated group.

naling involves cleavage of NICD by the  $\gamma$ -secretase complex (Nosedá et al., 2006). As an initial screen to identify optimal concentration of cocaine that induced increased expression of cleaved Notch1 (NICD), HBMECs were first treated with varying concentrations of cocaine (1, 10, and 100  $\mu$ mol/L) for 3 h and then assessed for induction of NICD by WB. As regards the physiological relevance of cocaine concentrations used in our study, it has been shown in humans that plasma cocaine concentrations following intranasal administration of cocaine often range between 0.4 and 1.6  $\mu$ mol/L (Van Dyke et al., 1976), while plasma cocaine concentrations in tolerant abusers reach levels of 13  $\mu$ mol/L (Stephens et al., 2004). Additionally, cocaine concentrations up to 100  $\mu$ mol/L and higher have been reported in post-mortem brains of chronic human cocaine users following acute intoxication (Kalasinsky et al., 2000). We thus reasoned that cocaine concentrations ranging from 1 to 100  $\mu$ mol/L would be compatible to the levels observed in humans.

As shown in Figure 1A, there was maximal induction of NICD at a concentration of 10  $\mu$ mol/L with slightly lower levels in cells exposed to 100  $\mu$ mol/L cocaine. The next step was to determine the time course of cocaine-mediated induction of NICD. As shown in Figure 1B, exposure of HBMECs to cocaine for varying times resulted in time-dependent induction of NICD with a maximal response at 3 h, followed by a gradual decline thereafter (Fig. 1B). As a control for Notch signaling pathway, the canonical Notch target signature genes *Hes1* and *Hes5* were also examined



**Figure 3.** CSL activation is necessary for cocaine-mediated induction of PDGF-B gene. **A,** RNA extracted from HBMECs transfected with either CSL/Non siRNAs were subject to RT-PCR analysis. **B,** CSL siRNA but not Non siRNA inhibited cocaine-mediated induction of PDGF-B. HBMECs were transfected with either CSL or Non siRNAs followed by treatment with cocaine (10  $\mu$ mol/L) for 12 h and assessed for PDGF-B expression. **C,** Transfection of cells with plasmid encoding CSL resulted in overexpression of CSL. **D,** CSL overexpression increased PDGF-B expression, and this was enhanced by cocaine (10  $\mu$ mol/L) treatment for 12 h. **E,** ChIP assay in HBMECs expressing Flag-tagged CSL or vector control. PCR with primers flanking the CSL consensus sequence in the PDGF-B promoter was performed following immunoprecipitation with the Flag antibody. Representative immunoblots and the densitometric analyses of PDGF-B/ $\beta$ -actin from four individual experiments are presented. All data are presented as mean  $\pm$  SD. \*\* $p$  < 0.01 versus control group; ## $p$  < 0.01 versus cocaine-treated group.

for increased transcription. As expected, HBMECs treated with cocaine (10  $\mu$ mol/L) for 3 h demonstrated a significant increase in *Hes1* and *Hes5* mRNA levels (Fig. 1C,D). Since activation of immediate Notch downstream target genes requires  $\gamma$ -secretase-mediated cleavage of NICD from the membrane, we next assessed the effect of  $\gamma$ -secretase inhibitor-DAPT on cocaine-mediated activation of Notch 1. HBMECs were pretreated with either 10 or 50  $\mu$ mol/L DAPT for 1 h followed by exposure to 10  $\mu$ mol/L cocaine for 3 h. As shown in Figure 1E, DAPT significantly suppressed cocaine-mediated upregulation of NICD, thereby underscoring the role of Notch1 signaling in HBMECs exposed to cocaine. DAPT alone failed to alter the basal levels of NICD expression (Fig. 1F).

### PDGF-B is a novel Notch1 immediate downstream target gene

It is well documented that members of the PDGF family play critical roles as cerebrovascular permeants, especially in BBB damage (Su et al., 2008). Furthermore, in our recent findings we have demonstrated that cocaine impaired endothelial integrity through PDGF-B (Yao et al., 2011a). To explore the relationship between cocaine-mediated activation of Notch 1 signaling and induction of PDGF-B expression, HBMECs were pretreated with DAPT and assessed for PDGF-B expression following 12 h treatment with cocaine. The concentrations used for cocaine exposure and the time point for PDGF-B expression were based on previous report (Yao et al., 2011a). HBMECs were pretreated with



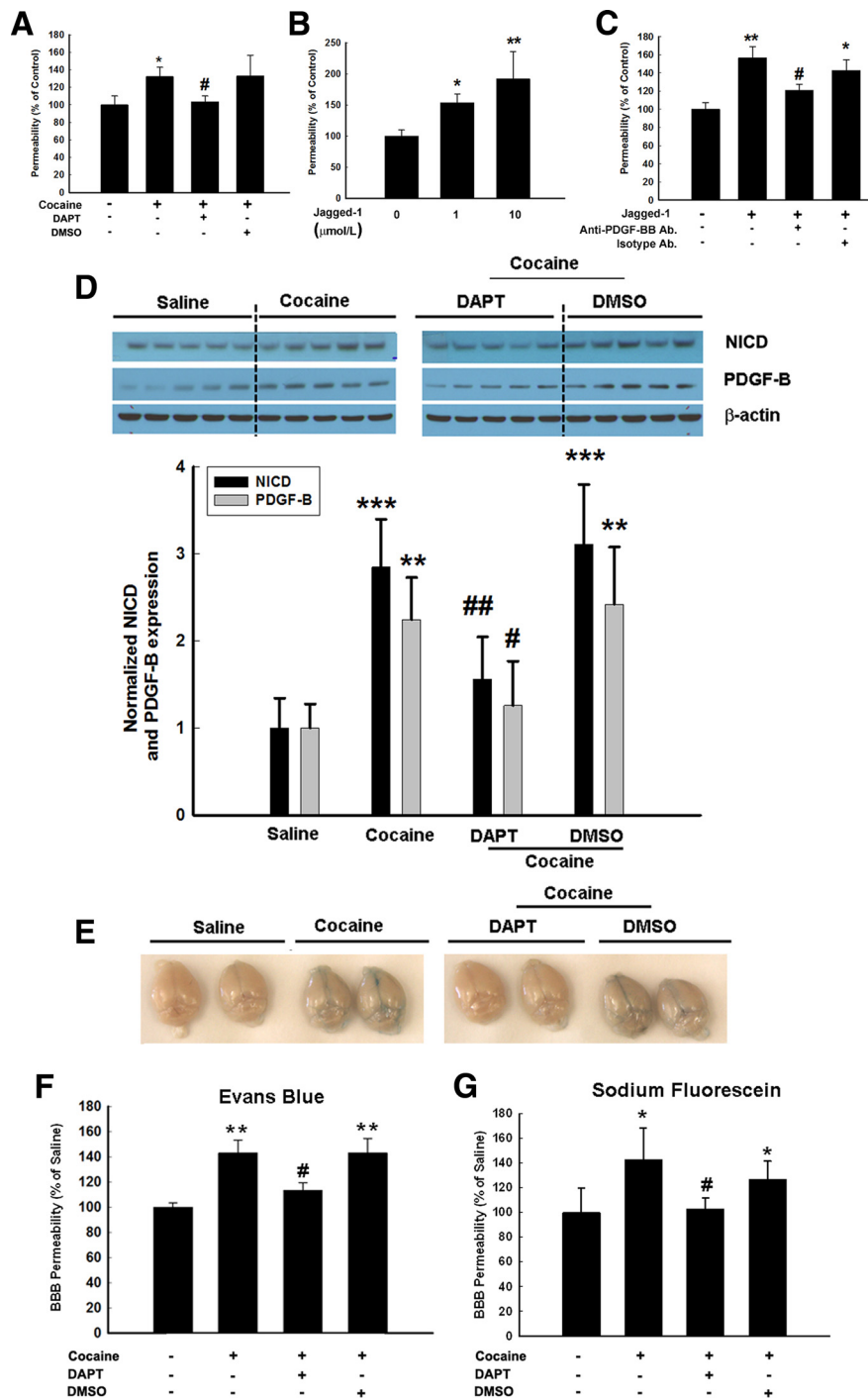
either 10 or 50  $\mu\text{mol/L}$  DAPT for 1 h followed by cocaine (10  $\mu\text{mol/L}$ ) exposure for 12 h. DAPT (50  $\mu\text{mol/L}$ ) significantly ameliorated cocaine-mediated PDGF-B expression (Fig. 2*A,B*), thus underpinning the role of Notch1 in this process. Next, we wanted to validate the role of Notch1 signaling in cocaine-mediated induction of PDGF-B gene using the gene silencing approach by transfecting HBMECs with siRNA Notch1. Transfection of HBMECs with Notch1 siRNA resulted in efficient knockdown of Notch1 protein (Fig. 2*C*). Intriguingly, Notch1 siRNA but not the nonsense (Non) siRNA significantly abrogated cocaine-mediated upregulation of PDGF-B expression (Fig. 2*D,E*).

To further confirm the role of Notch1 signaling in induction of PDGF-B, an alternative approach such as treating HBMECs with a known Notch1 ligand (Jagged-1 peptide) was used. As shown in Figure 2*F*, Jagged-1-mediated activation of Notch1 was capable of inducing NICD as expected, and also PDGF-B in HBMECs. Reciprocally, overexpressing NICD in HBMECs by transfecting cells with the NICD construct also led to upregulation of PDGF-B (Fig. 2*G*), thereby leading to the suggestion that PDGF-B gene was a downstream target of Notch1 in HBMECs.

#### CSL activation is necessary for cocaine-mediated induction of PDGF-B

Based on the fact that NICD interacts with CSL to initiate gene transcription, and since human PDGF-B promoter contains a CSL consensus-binding site (TGGGAA), we enquired whether CSL activation was sufficient to induce PDGF-B expression. To achieve this, HBMECs were transfected with either a siRNA-CSL or a CSL overexpression construct and exposed to cocaine, following which PDGF-B chain expression was monitored 12 h after treatment. Knockdown of CSL was confirmed by RT-PCR (Fig. 3*A*). Intriguingly, cocaine failed to increase PDGF-B expression in cells transfected with CSL siRNA compared with cells transfected with a Non siRNA (Fig. 3*B*). Validation of the role of CSL in Notch1-mediated expression of PDGF-B was also confirmed in cells overexpressing the CSL construct. In these cells, as expected, there was increased expression of PDGF-B, which was enhanced in the presence of cocaine at 12 h (Fig. 3*C,D*).

The next step was to confirm the binding of CSL to the PDGF-B promoter. For this, chromatin immunoprecipitation assay was performed in HBMECs transfected with vector encoding Flag-tagged



**Figure 4.** Notch1 signaling is critical for cocaine-mediated disruption of BBB. **A**, Cocaine-mediated increase in BBB permeability was ameliorated in HBMECs pretreated with DAPT (50  $\mu\text{mol/L}$ ). **B**, Jagged-1 (1 or 10  $\mu\text{mol/L}$ ) increased BBB permeability. **C**, Pretreatment of HBMECs with the PDGF-B-neutralizing antibody ameliorated Jagged-1 (10  $\mu\text{mol/L}$ )-induced BBB permeability. All the data are presented as mean  $\pm$  SD of four individual experiments. \* $p$  < 0.05, \*\* $p$  < 0.01 versus control group; # $p$  < 0.05 versus cocaine-treated group. **D**, Cocaine administration in mice resulted in increased expression of NICD and PDGF-B, which was ameliorated by pretreatment with DAPT, but not with DMSO. Representative immunoblots and the densitometric analyses of NICD/ $\beta$ -actin and PDGF-B/ $\beta$ -actin from five animals per group are presented. \*\*\* $p$  < 0.01; \*\*\*\* $p$  < 0.001 versus control group; # $p$  < 0.05; ## $p$  < 0.01 versus cocaine-treated group. **E**, Dissected brains from saline, cocaine, cocaine + DAPT, and cocaine + DMSO groups of animals that were injected IV with Evans blue dye. Note the uniform bluish color in the brains of cocaine and cocaine + DMSO groups, which was absent in saline and cocaine + DAPT groups. Quantification of Evans blue (**F**) and sodium fluorescein (**G**) extravasation into the brains following cocaine injection. All the data are presented as mean  $\pm$  SD,  $n$  = 5 per group. \* $p$  < 0.05, \*\* $p$  < 0.01 versus saline group; # $p$  < 0.05 versus cocaine-treated group.

CSL or an empty vector. As shown in Figure 3E, PCR of Flag-immunoprecipitated DNA using primers flanking the CSL consensus site confirmed direct binding of CSL to the PDGF-B promoter.

### Notch1 signaling is critical for cocaine-mediated disruption of BBB

Having determined PDGF-B as a novel target gene of Notch1 signaling and keeping in mind that PDGF-B is a known vascular permeant (Yao et al., 2011a), we next sought to examine whether cocaine-mediated Notch1 signaling played a critical role in mediating increased permeability of endothelial cells. Exposure of HBMEC monolayer to cocaine resulted in increased permeability, which was ameliorated in cells pretreated with DAPT (Fig. 4A). Intriguingly, Jagged-1 peptide was also capable of enhancing endothelial cell permeability (Fig. 4B), and this effect was ameliorated by PDGF-B-neutralizing antibody (Fig. 4C). These findings thus underscore the role of Notch1 signaling in cocaine-mediated enhancement of endothelial permeability.

### In vivo involvement of Notch1 signaling in cocaine-mediated permeability

To validate cocaine-mediated induction of NICD *in vivo*, brain homogenates isolated from cocaine versus saline-treated mice were assessed for expression of both NICD and PDGF-B. As shown in Figure 4D, cocaine-administered mice demonstrated increased expression of both NICD and PDGF-B in brain tissues compared with saline group. Interestingly, mice pretreated with DAPT demonstrated attenuated expression of both NICD and PDGF-B compared with cocaine-treated animals. DMSO-treated animals, as expected, demonstrated cocaine-mediated induction of NICD and PDGF-B.

Having determined that cocaine treatment enhanced expression of its downstream targets, NICD and PDGF-B, the next step was to carry out functional assays to determine whether mice treated with cocaine demonstrated increased permeability of BBB. We evaluated the extravasation of Evans blue dye into the brain parenchyma, an indicator of disrupted BBB permeability (Armulik et al., 2010; Daneman et al., 2010). In the cocaine-treated group, there was increased Evans blue extravasation into the brain parenchyma compared with the saline group, and this effect was ameliorated by mice pretreated with DAPT, but not DMSO. Quantification of the Evans blue dye in the brain parenchyma demonstrated enhanced levels in cocaine-treated group compared with the DAPT-treated group. As expected, no Evans blue extravasation was observed in saline-treated mice (Fig. 4E,F). To further validate this finding, permeability of low-molecular-weight sodium fluorescein was also assessed in the brains of cocaine- and saline-treated mice. As demonstrated in Figure 4G, cocaine administration resulted in increased vascular permeability compared with the DMSO group, and this effect was ameliorated in mice pretreated with DAPT, lending support to the suggestion that Notch 1 signaling plays a critical role in vascular leakiness.

### Discussion

In summary, we have identified PDGF-B as a novel Notch1 downstream target gene in endothelial cells. While there are reports on the role of Notch1 signaling in neurogenesis (Wang et al., 2004) and synaptic plasticity (Berezovska et al., 1999), our findings suggest yet another function of Notch1 signaling—that of mediating increased endothelial permeability via induction of PDGF-B expression. Cocaine has been shown to impair BBB by

modulating transcriptional regulation of key cellular functional genes (Fiala et al., 1998). This study highlights yet another gene, PDGF-B that is regulated by cocaine and its downstream Notch1 signaling.

In this study, using both the pharmacological inhibitor of  $\gamma$ -secretase DAPT and the siRNA-mediated knockdown of NICD, we validated the role of cocaine-mediated activation of Notch in PDGF-B expression. While these knockdown approaches are valuable tools, it must be pointed out that they are not mutually exclusive and need to be used in conjunction to confirm the role of Notch pathway. For example, using DAPT, we did not achieve complete abrogation of PDGF-B (Fig. 2B), unlike the siRNA NICD-transfected cells (Fig. 2E) that demonstrated a complete knockdown of PDGF-B. Since at the present time there are no reagents available to measure the  $\gamma$ -secretase activity, it could be speculated that either the incomplete inhibition of PDGF-B by DAPT could be attributed to an incomplete blockade of  $\gamma$ -secretase or that there must exist a  $\gamma$ -secretase-independent compensatory pathway upstream of NICD that could be involved in activation of NICD and hence induction of PDGF-B.

This study also addresses an important issue of how drugs of abuse, such as cocaine, can hijack host intracellular signaling pathways to achieve cellular dysfunction. This is the first study to elucidate cocaine-mediated activation of Notch1 signaling. These findings could have ramifications for the role of Notch1 signaling in addiction research in HIV-infected individuals.

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