Dopamine in vivo inhibits VEGF-induced phosphorylation of VEGFR-2, MAPK, and focal adhesion kinase in endothelial cells

Chandradi Sarkar,1 Debanjan Chakroborty,1 Rita Basu Mitra,3 Samir Banerjee,1 Partha Sarathi Dasgupta,1 and Sujit Basu2,4

1Signal Transduction and Biogenic Amines Laboratory and 2Department of Medical Oncology, Chittaranjan National Cancer Institute, Calcutta 700026; 3Department of Pathology, Institute of Postgraduate Medical Education and Research, Calcutta 700020, India; and 4Mayo Clinic Cancer Center and Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, Minnesota 55905

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TRIGGERING THE ANGIOGENIC switch is an important event in several physiological and pathological processes (6, 8). In animals, including humans, these processes operate as a shift in the balance between production of endogenous molecules that either stimulate or inhibit angiogenesis (4). Among the several stimulators of angiogenesis, the vascular permeability factor (VPF)/VEGF is thought to be the single most important angiogenic cytokine (6, 8). Thus understanding the endogenous regulators of VPF/VEGF-mediated angiogenesis in vivo has become an emerging area of research (9, 13). Because pharmacologically administered dopamine cannot cross the blood-brain barrier (12), our (S. Basu and P. S. Dasgupta, unpublished observations) recent results indicated that a nontoxic pharmacological dose of dopamine can significantly and specifically inhibit VPF/VEGF-mediated microvascular permeability, proliferation, and migration of cultured endothelial cells in vitro (3). Therefore, we reasoned that the endogenous neurotransmitter dopamine might regulate the important signaling cascades mediating in vivo endothelial functions of VEGF. Because VEGF-induced phosphorylation of VEGF-receptor 2 (VEGFR-2), focal adhesion kinase (FAK), and MAPK (5, 7, 10, 17, 18, 31) are critical for these activities of VPF/VEGF, we therefore investigated whether endogenous dopamine has any role in inhibiting these important signaling events.

MATERIALS AND METHODS

Mice and reagents. Four- to six-week-old male C57 BL/6 mice were obtained from the institutional animal facility and male C57BL/6 dopamine D2 receptor knockout mice of the same age range were supplied by Jackson Laboratories. The 4- to 6-wk-old male Swiss mice were supplied by the institutional animal facility. The mice were caged in plastic tubs covered with stainless steel tops at a temperature between 23 and 25°C, humidity level of 50 ± 10%, and a 12:12-h light-dark cycle. These animals were given ad libitum water and rodent chow. All procedures in animals were approved by the institutional animal care and use committee. 1-Methyl-4-phenylpyridinium iodide (MPP+) iodide, 2-2-2-tetrabromoethanol (Avertin), quinpirole, domperidone, phenolamine, and propranolol were obtained from Sigma (St. Louis, MO). Collagenase and DNase were from Roche Diagnostics (Indianapolis, IN). R-phcoerythrin-conjugated rat anti-VEGFR-2 (catalog no. 555308), rat anti-CD 31 (catalog no. 553373), and CD34 (catalog no. 551387) monoclonal antibodies for flow cytometry analysis were from BD Biosciences Pharmingen (San Diego, CA). Rat anti-CD 31 (catalog no. 558736) for immunohistochemistry was purchased from BD Biosciences Pharmingen. VEGFR-2 antibody (catalog no. sc-6251), phospho-MAPK antibody (catalog no. sc-93), and dopamine D2 receptor antibody (catalog no. sc-9113) was from Santa Cruz Biotechnology (Santa Cruz, CA). Phosphotyrosine antibody (catalog no. 05-321) was purchased from Upstate Biotechnology (Lake Placid, NY), and monoclonal antibodies against FAK (catalog no. MAB2156) were from Chemicon and used for immunoprecipitation and immunoblotting. The ABC kit was from Vector Labs (Burlingame, CA) and recombinant human VPF/VEGF was from R&D systems (Minneapolis, MN). The RT-PCR kit was purchased from Ambion.

Depletion of endogenous dopamine. The endogenous dopamine in these syngeneic mice were selectively depleted by subcutaneous injection of neurotoxin, MPP+ iodide at a dose of 10 mg/kg for 4 continuous days (11), causing >90% depletion of dopamine (11, 16).

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Address for reprint requests and other correspondence: S. Basu, Mayo Clinic Cancer Center and Dept. of Biochemistry and Molecular Biology, 200 First St. SW; Gugg. 1494, Mayo Clinic, Rochester, MN 55905 (E-mail: basu.sujit@mayo.edu); or P. S. Dasgupta, Signal Transduction and Biogenic Amines Laboratory, Chittaranjan National Cancer Institute, 37 S. P. Mukherjee Rd., Calcutta 700026, India (E-mail: partha2@vsnl.com).
In addition, this neurotoxin was particularly selected because it has no direct effect on microvessel permeability (assessed by Miles assay) or angiogenesis (assessed in the peritoneal lining) when injected to normal mice (data not shown). Further experiments were then undertaken in these dopamine-depleted mice 24 h after the last dose of MPP⁺ iodide injection.

Collection of endothelial cells from mesentery by fluorescence-activated cell sorting. Microvascular endothelial cells (MVEC) were chosen because these cells were reported to be highly responsive to intraperitoneal VPF/VEGF injection (19, 20). These cells were isolated from the mesentery of normal and dopamine-depleted mice. Furthermore, the mesenterial endothelial cells were also isolated from normal mice treated with the specific dopamine D₂ receptor agonist quinpirole (10 mg/kg ip), or the antagonist domperidone (10 mg/kg ip), the specific α-blocker phenotolamine (5 mg/kg sc) or the specific β-blocker propranolol (15 or 25 mg/kg ip) 15 min before VPF/VEGF injection. Briefly, normal mice, dopamine-depleted mice, and mice previously treated with either D₂ receptor agonist or antagonist or adrenergic antagonists were injected intraperitoneally with 500 ng of recombinant human VPF/VEGF in 1 ml of HBSS. These animals were then anesthetized with an injection of 0.4–0.75 mg/g ip Avertin and were euthanized by decapitation. The cleansed abdomen was opened, and the heart was perfused with 10 ml of PBS to remove circulating blood cells from the mesenteric vessels. The intestine was isolated from the abdominal cavity and the mesentery was removed. The entire procedure for removal of mesentery from the animal took 4 min after injection of VPF/VEGF. Furthermore, the mesentery thus removed was digested with collagenase and DNase in DMEM for 3 h at 37°C injection of VPF/VEGF. Brieﬂy, normal mice, dopamine-depleted mice, and mice previously treated with either D₂ receptor agonist or antagonist or adrenergic antagonists were injected intraperitoneally with 500 ng of recombinant human VPF/VEGF in 1 ml of HBSS. These animals were then anesthetized with an injection of 0.4–0.75 mg/g ip Avertin and were euthanized by decapitation. The cleansed abdomen was opened, and the heart was perfused with 10 ml of PBS to remove circulating blood cells from the mesenteric vessels. The intestine was isolated from the abdominal cavity and the mesentery was removed. The entire procedure for removal of mesentery from the animal took 4 min after injection of VPF/VEGF. Furthermore, the mesentery thus removed was digested with collagenase and DNase in DMEM for 1 h at 37°C. The resulting suspension was ﬁltered. Cell suspensions were then labeled with phycocerythrin-conjugated anti-VEGFR-2 (1:100), anti-CD31 (1:100), and anti-CD34 (1:100). One million positive endothelial cells were collected by ﬂuorescence-activated cell sorting (FACSCalibur, BD Biosciences; San Jose, CA) (22).

Semiquantitative RT-PCR. From isolated endothelial cells, total RNA was isolated with an RNA isolation kit (Ambion). Total RNA (2 μg) was reverse transcribed into ﬁrst-strand cDNA with the use of poly-dT priming, and a 10-μl cDNA product was used for PCR ampliﬁcation with the use of 1× PCR buﬀer (Ambion). Then, 2.0 units of superTag DNA polymerase (Ambion) and 1 μl of upstream and downstream primer of dopamine D₂ receptor were added to create a ﬁnal volume of 40 μl. PCR was carried out in a DNA thermocycler (Gene-Amp-9700; Applied Biosystem) after ﬁrst denaturation at 94°C for 3 min, and each cycle consisted of denaturation at 94°C for 40 s, annealing at 59°C for 40 s, and extension at 72°C for 80 s. The number of total cycle was 37. In this experiment, S15RNA was served as control. This control PCR primer ampliﬁes a 361 bp, highly conserved region of a highly conserved region of a housekeeping gene, rig/S15, which encodes for a small ribosomal subunit protein (14). Because it is a constitutively expressed message, S15RNA is present in any cells. PCR for S15RNA was carried out for 27 cycles and each cycle consisted of denaturation at 94°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 1 min. The obtained PCR products were veriﬁed by sequence analysis from the institute sequencing facility. For quantitative analysis, PCR products were run on 2% agarose gel, stained with ethidium bromide, and quantitation was performed with the use of a densitometer with image analysis software (Pharmacia). The sequences of PCR primers for S15 (control) and dopamine D₂ receptor were as follows: S15 (Internal Control), 5'-TTCCGAAGTCTACCTAC-3' (14); 5'-GGGCGGGCGCCCAT-GCTTTACG-3' (14); dopamine D₂ receptor, 5'-GACCGCCAGTC-TCAGGGGC-3'; 5'-GGTAGTGTTGCGTCAGCT-3' (GenBank Accession No. S69899).

Immunoprecipitation and immunoblotting. Cell lysates were immunoprecipitated with different antibodies (1:100 dilution, except phospho-MAPK antibody was used at 1:1,000 dilution), and immunocomplexes were captured on protein A-agarose beads. Samples were subjected to SDS-PAGE and then transferred to polyvinyl difluoride membranes and immunoblotted. Antibody-reactive bands were detected by enzyme-linked chemiluminescence (Amersham) and quantiﬁed by laser densitometry.

Ascitic ﬂuid volume measurement and immunohistochemistry. Tumor ascites is largely attributable to increased permeability of peritoneal lining vessels, and microvascular hyperpermeability is an early and consistent step in the angiogenic cascade initiated by VPF/VEGF (1, 7, 20, 26). Therefore, we determined the ascites ﬂuid volume and angiogenic response in peritoneal lining tissues of sarcoma 180 (S180)-bearing mice, a well-characterized mouse tumor, which secretes VPF/VEGF (1, 15, 25, 30). Brieﬂy, both normal and dopamine-depleted mice were injected intraperitoneally with 1 × 10⁶ S180 cells. Then, on day 7 after tumor transplantation, the ascite ﬂuid volume was determined in these animals. Furthermore, to determine the angio- genic response in the peritoneal lining tissues, the S180-bearing mice were euthanized on day 7 by CO₂ narcosis, and the parietal peritoneum was then ﬁxed in 4% paraformaldehyde, rinsed in PBS, and transferred to 30% sucrose in PBS, and frozen in OCT compound. From these frozen tissue samples, 12-μm-thick sections were cut and collected on to a poly-l-lysine-coated glass slide. Sections were then ﬁxed in aceton at −20°C for 30 min and stored in −80°C. For immunohistochemistry, sections were ﬁrst air dried for 5 min at room temperature and incubated with the primary antibody (1:50) against CD 31, and then the ABC staining kit was used. Microvessel density was quantitated by analyzing 10 random ﬁelds per section (23). Similarly, the angiogenic response in the parietal peritoneum of dopamine-depleted and dopamine-intact mice with no tumors was determined.

Miles assay. Both dopamine-nondepleted and dopamine-depleted Swiss mice with no tumors received intravenous injections of 200 μl of Evans blue dye (0.5%) via the tail vein immediately before intradermal skin test with 50 μl of VPF/VEGF (25 ng) or vehicle (HBSS). The leakage of the dye was measured by calculating the two dimensions (a

![Image](http://ajpheart.physiology.org/)

Fig. 1. A: semiquantitative RT-PCR shows D₂ class of dopamine receptor mRNA in microvascular endothelial cells (MVEC) and in positive control human umbilical vein endothelial cells (HUVEC) but not in negative control sarcoma 180 (S180) tumor cells. Results are representatives of six separate experiments with similar results. B: immunoblot shows ~85-kDa D₂ dopamine receptor in extracts of MVEC and in positive control HUVEC but not in negative control S180 tumor cells. The figure is representatives of six separate experiments with similar results.
and b) of the elliptically appearing area of dye leakage after 30 min, which was proportional to the vascular permeability (3, 26, 27, 32), and the area was calculated with the formula \( a \times b \times \pi \) (27).

Densitometric quantitation of vascular permeability. Densitometric analysis was performed with the use of NIH Image Analysis software (version 1.62; Bethesda, MD) as another means of quantifying the extent of dye leakage at the intradermal injection sites in each mouse. Digitally obtained images of the dorsal skin, including all injection sites, were converted to a grayscale image, and dye density was analyzed at each site: the threshold was set individually for each dorsal skin flap but was constant for each mouse (27).

**Fig. 2.** Increased vascular permeability factor (VPF)/VEGF-induced phosphorylation of VEGF receptor 2 (VEGFR-2) (A), focal adhesion kinase (FAK) (B), and MAP kinase (C) in endothelial cells collected from the mesentery of VEGF-treated dopamine-depleted (DEP+VEGF) mice (lane 3) compared with VEGF-treated non-dopamine-depleted (N + VEGF) control (lane 2). Lane 1 represents baseline phosphorylation (VEGF-untreated normal mice). The bar graphs represent the density of each phosphorylated protein band relative to the basal phosphorylation level of each molecule in control group (VEGF-untreated normal mice: lane 1 in the bar graph) as quantified by laser densitometry. IP, immunoprecipitate; PTyr, phosphotyrosine; KDR, kinase domain receptor. *P < 0.05 compared with basal value. Results are representatives of six separate experiments, each yielding similar results.

**Fig. 3.** Decreased VPF/VEGF-induced phosphorylation of VEGFR-2 (A), FAK (B), and MAP kinase (C) in endothelial cells collected from the mesentery of VEGF-treated normal mice injected before with dopamine D2 receptor agonist quinpirole (N + QUIN + VEGF; lane 3) and increased VPF/VEGF-induced phosphorylation of VEGFR-2, FAK, and MAP kinase in endothelial cells collected from the mesentery of VEGF-treated normal mice injected previously with dopamine D2 receptor antagonist domperidone (N + DOM + VEGF; lane 4) compared with VEGF-treated normal control (N + VEGF; lane 2). Mice treated previously with the \( \alpha \)-blocker phentolamine (N + PHE + VEGF; lane 5) and the \( \beta \)-blocker propranolol (N + PROP + VEGF; lane 6) show no change compared with VEGF-treated normal control (N + VEGF). The bar graphs represent the density of each phosphorylated protein band relative to the basal phosphorylation level of each molecule in control group (VEGF-untreated normal mice: lane 1 in the bar graph) as quantified by laser densitometry. *P < 0.05 compared with basal value; +P < 0.05 compared with VEGF-induced phosphorylation. Results are representatives of six separate experiments, each yielding similar results.

**Statistics.** Differences among groups were evaluated by ANOVA and the unpaired Student’s t-test or Dunn’s multiple-comparison test. Statistical significance was assigned when \( P < 0.05 \).

**RESULTS**

Dopamine D2 receptors are present on mesenteric endothelial cells. Because it has been previously reported (3) that dopamine inhibits the actions of VPF/VEGF by acting on the D2 receptors present on the cultured endothelial cells in vitro, we therefore initially performed experiments to deter-
mine whether dopamine D$_2$ receptors are present on the endothelial cells collected from the mesentery. Dopamine D$_2$ receptor mRNA (Fig. 1A) and proteins (Fig. 1B) were found to be expressed in these endothelial cells. VPFF/VEGF-induced phosphorylation of VEGFR-2 and MAPK was increased in endothelial cells collected from mesenteries of dopamine-depleted mice. Because phosphorylation of VEGFR-2 and MAPK is critical for VPFF/VEGF-mediated activities of endothelial cells (5, 10, 18, 31), and phosphorylation of FAK is associated with VPFF/VEGF-induced microvascular permeability (17, 19), we therefore elucidated the effects of modulation of endogenous dopamine in controlling these important signaling events. VPFF/VEGF-induced phosphorylation of VEGFR-2 was markedly increased in endothelial cells collected from dopamine-depleted mice compared with endothelial cells collected from normal mice (Fig. 2A). Similarly, VPFF/VEGF-mediated phosphorylation of FAK (Fig. 2B) and MAPK (Fig. 2C) were increased in these cells compared with normal controls. The bar graphs represent the density of each phosphorylated protein band relative to the basal phosphorylation level of each molecule in control group (VPFF/VEGF untreated normal mice: lane 1 in the bar graph) as quantified by laser densitometry. *P < 0.05 compared with basal value. Results are representative of six separate experiments, each yielding similar results.

Fig. 4. Increased VPFF/VEGF-induced phosphorylation of VEGFR-2 (A), FAK (B), and MAP kinase (C) in endothelial cells collected from the mesentery of VEGF-treated dopamine D$_2$ receptor knockout mice [D$_{D2}^{+/−}$ + VEGF, lane 3] compared with VEGF-treated normal control (N + VEGF, lane 2). The bar graphs represent the density of each phosphorylated protein band relative to the basal phosphorylation level of each molecule in control group (VEGF untreated normal mice: lane 1 in the bar graph) as quantified by laser densitometry. *P < 0.05 compared with basal value. Results are representatives of six separate experiments, each yielding similar results.

Fig. 5. Increased ascites fluid accumulation (A) in S180-bearing dopamine-depleted mice (Dep+S180) compared with S180-bearing non-dopamine-depleted controls (S180). In the Miles assay, both gross and densitometric analysis of dorsal skin (B and C) show depletion of dopamine (Dep+VEGF) increases the vascular permeability enhancing activity of VPFF/VEGF compared with non-dopamine-depleted controls (Normal+VEGF). There is also no increase in the permeability in VPFF/VEGF untreated dopamine-depleted (Dep+HBSS) group when compared with VPFF/VEGF untreated non-dopamine-depleted (Dep+HBSS) controls. The blue spots (measured at 30 min) reflect leakage of Evans blue dye plasma protein complexes, and are proportional to the extent of vascular permeability. *P < 0.05, results are representative of 6 separate experiments, each yielding similar results.
(Fig. 2C) were also strikingly increased in endothelial cells collected from dopamine-depleted mice than endothelial cells collected from normal animals. Together, these results clearly indicated that endogenous dopamine is a physiological regulator of VPF/VEGF-mediated activities of endothelial cells.

Dopamine D2 receptors modulate VPF/VEGF-induced phosphorylation of VEGFR-2, FAK, and MAPK. We next investigated whether the action of endogenous dopamine is specific. Intraperitoneal injection of specific dopamine D2 receptor agonist quinpirole 15 min before VPF/VEGF injection, caused striking decrease in VPF/VEGF-induced phosphorylation of VEGFR-2 (Fig. 3A), FAK (Fig. 3B), and MAPK (Fig. 3C). In contrast, although blockade of dopamine D2 receptors by intraperitoneal injection of the specific D2 receptor antagonist domperidone 15 min before VPF/VEGF injection caused a marked increase in VPF/VEGF-induced phosphorylation of VEGFR-2 (Fig. 3A), FAK (Fig. 3B), and MAPK (Fig. 3C), α- and β-adrenergic blockers showed no changes (Fig. 3, A–C), thereby indicating that endogenous dopamine specifically regulates the actions of VPF/VEGF on endothelial cells in vivo through the dopamine D2 receptors present on these cells. Like quinpirole and domperidone, similar results were also observed when mice were treated with other specific D2 receptor agonists and antagonists (results not shown).

Knockout of dopamine D2 receptor in vivo increases VPF/VEGF-induced phosphorylation of VEGFR-2, FAK, and MAPK. Intraperitoneal injection of VPF/VEGF into dopamine D2 receptor knockout mice caused striking increase in the phosphorylation of VEGFR-2 (Fig. 4A), FAK (Fig. 4B), and MAPK (Fig. 4C), thereby confirming that the actions of VPF/VEGF in vivo is controlled by endogenous dopamine through D2 receptors present on the endothelial cells.

Malignant ascites formation and peritoneal angiogenesis is increased in S180-bearing dopamine-depleted mice. The volume of malignant ascites fluid (Fig. 5A) was significantly increased in dopamine-depleted S180-bearing mice compared with S180-bearing mice with intact dopamine, thereby indicating that endogenous dopamine may have a role in modulating VPF/VEGF-induced vascular permeability. Our result was confirmed by the Miles assay, a sensitive method for assessing vascular permeability (3, 26, 27, 32). We found that, although depletion of endogenous dopamine had no direct effect on vascular permeability in mice with no tumors, there was a striking increase in VPF/VEGF-induced vascular permeability in these animals compared with dopamine-intact controls (Fig. 5, B and C). Furthermore, the angiogenic response in the peritoneal lining tissue also was significantly increased in dopamine-depleted S180-bearing mice compared with S180-bearing mice with intact dopamine (Fig. 6, A–E). It would be noteworthy to mention here that depletion of dopamine had no direct effect on angiogenesis because we did not find any difference in angiogenesis between dopamine-depleted and dopamine-intact mice with no tumors (Fig. 6, A and C). These results thus indicate that endogenous dopamine may have a role in modulating VPF/VEGF angiogenesis in vivo.

DISCUSSION

Taken together, our results indicate that endogenous neurotransmitter dopamine by acting through dopamine D2 receptors modulates VPF/VEGF-mediated vascular permeability and angiogenesis by inhibiting phosphorylation of VEGFR-2, FAK, and MAPK in endothelial cells in vivo. This finding is significant because phosphorylation of VEGFR-2 is critical for VPF/VEGF-mediated microvascular permeability, endothelial cell proliferation, and migration (5, 7, 10, 18, 31). Furthermore, VPF/VEGF-mediated phosphorylation of FAK is associated with increased microvascular permeability (17, 19), an essential first step in both physiological and pathological angiogenesis (6) and VPF/VEGF-mediated MAPK phosphorylation is critical for endothelial cell proliferation (10, 18, 31).

We had previously reported that dopamine at a nontoxic pharmacological dose can inhibit VPF/VEGF-mediated activities of cultured human umbilical vein endothelial cells in vitro. However, in that report, the actions of pharmacological dose of dopamine were elucidated in cultured human umbilical vein endothelial cells, where those cells were not subjected to the regulatory mechanisms that may be imposed in vivo by, i.e.,
neighboring cells (e.g., pericytes), flowing blood, and vascular pressure (19). Here, we have demonstrated for the first time that the endogenous neurotransmitter dopamine is a potent regulator of the three important signaling cascades, thus modulating actions of VPF/VEGF on endothelial cells in vivo and hence angiogenesis. Our results also provide new mechanistic insight into the dopamine-mediated inhibition of the activities of VPF/VEGF.

Furthermore, our present result is important because there are reports which indicate that endogenous dopamine may regulate malignant tumor growth (2, 28), and therefore it will be interesting to suggest here that endogenous dopamine might regulate tumor growth by modulating VPF/VEGF-mediated angiogenesis in vivo. It would also be noteworthy to mention that we had recently observed absence of dopamine D_2 receptor expression in fetal peripheral blood vessels (S. Basu and P. S. Dasgupta; unpublished observations) and therefore dopamine would not be expected to have any deleterious effects on VEGF-mediated developmental angiogenesis. Indeed, there are some reports (21, 24, 29) that indicate that administration of dopamine D_2 receptor agonists during pregnancy in rats (prolactin-independent phase) and humans is devoid of any angiogenesis in vivo. It would also be noteworthy to suggest here that endogenous dopamine might be interesting to suggest here that endogenous dopamine might regulate tumor growth by modulating VPF/VEGF-mediated angiogenesis in vivo. It would also be noteworthy to mention that we had recently observed absence of dopamine D_2 receptor expression in fetal peripheral blood vessels (S. Basu and P. S. Dasgupta; unpublished observations) and therefore dopamine would not be expected to have any deleterious effects on VEGF-mediated developmental angiogenesis. Indeed, there are some reports (21, 24, 29) that indicate that administration of dopamine D_2 receptor agonists during pregnancy in rats (prolactin-independent phase) and humans is devoid of any angiogenesis in vivo. It would also be noteworthy to mention that we had recently observed absence of dopamine D_2 receptor expression in fetal peripheral blood vessels (S. Basu and P. S. Dasgupta; unpublished observations) and therefore dopamine would not be expected to have any deleterious effects on VEGF-mediated developmental angiogenesis.

Finally, in addition to elucidating new mechanisms for endogenous dopamine-mediated regulation of VPF/VEGF activities in vivo, this study also suggests that the endogenous dopaminergic system may play a significant role in several physiological and pathological conditions in which VEGF-mediated angiogenesis has an important role.

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REFERENCES


26. Tennis MA, Kavelaars A, Voest E, Bakker JM, Ellenbroek BA, Cools AR, and Heijnjen CJ. Reduced tumor growth, experimental metastasis


