Control of Vascular Tone by Endogenous Endothelin-1 in Human Pial Arteries

Eric Thorin, PhD; Thanh-Dung Nguyen, BSc; Alain Bouthillier, MD

Background and Purpose—Endothelin-1 (ET) has been shown to be involved in human pathological conditions, but its physiological function remains to be elucidated. The aim of this work was to assess whether endothelium-derived ET was involved in the overall responsiveness of freshly isolated human pial arteries.

Methods—Samples of cerebral cortex, otherwise discarded, were obtained during tumor or epileptic lesion resections (n=10 donors). Arterial segments were isolated and mounted on a microvessel myograph.

Results—Inhibition of nitric oxide (NO) formation with Nω-nitro-L-arginine (L-NA, 100 μmol/L) increased basal tone by 7±1%Emax (n=5). This increase in tone was fully abolished in the presence of BQ123 (1 μmol/L; ETα receptor antagonist, P<.05) but potentiated by a subthreshold concentration of exogenous ET (1 nmol/L; 33±8%Emax; P<.05). In the presence of L-NA, serotonin (10 μmol/L)–induced tone was doubled compared with the control response (P<.05) but reduced by 90% in the presence of BQ123 (P<.05). In the absence of L-NA, BQ123 prevented serotonin–induced tone (n=3). Oxymetazoline, a selective α1-adrenergic receptor agonist, induced an endothelium-dependent relaxation of preconstricted human pial arteries. The relaxation was partially sensitive to NO synthase inhibition and fully prevented by the addition of ET, whereas substance P–induced relaxation was preserved. Glibenclamide (1 μmol/L), an inhibitor of ATP-sensitive K+ channels and tetraethylammonium (1 mmol/L), an inhibitor of Ca2+-activated K+ channels had no effect on oxymetazoline–induced relaxation.

Conclusions—The results of this study suggest first that ET is involved in the tonic response induced by NO synthase inhibition; second, part of the contractile response induced by serotonin is endothelium-dependent and sensitive to BQ123; and third, the data suggest that activation of α1-adrenergic receptors generated an endothelium-dependent relaxation that was selectively inhibited by exogenous ET. (Stroke. 1998;29:175-180.)

Key Words: endothelins • endothelium • nitric oxide synthase • pial arteries

Endothelin-1 is a potent endothelium-derived constricting factor first identified in the medium of cultured endothelial cells. It is a small peptide (21 amino acids) in which secretion is regulated by numerous factors. Most stimuli (such as α-thrombin, insulin, oxidized low-density lipoprotein, and hemodynamic shear stress) regulate ET release at the level of gene transcription. The expression of preproET mRNA is regulated by mechanisms that involve receptor-mediated mobilization of Ca2+ and activation of protein kinase C in endothelial cells. Secretion of ET is also calcium-dependent. Serotonin and angiotensin II have an immediate effect on ET release in various resistance arteries. PreproET and ET are stored in intracellular vesicles of cultured bovine aortic endothelial cells, suggesting that this could be a target for some stimuli.

The involvement of ET in the control of the cerebrovascular tone is uncertain. The reactivity of small arteries is regulated by numerous factors, including membrane potential, pressure, and shear stress as well as factors released by the endothelium. In cerebral arteries, all these factors are associated with the regulation of cerebral blood flow. A recent report showed that the diameter of canine cerebral arteries was enhanced 24 hours after in vivo injection of BQ123, an ETα receptor antagonist.

However, it is accepted that ET is critical in pathological states only. ET-1 levels increase in patients with subarachnoid hemorrhage and coronary heart disease. Antagonism of ET receptors successfully prevented the appearance of cerebral vasospasms and the development of early atherosclerotic lesions in animal models. However, there is no direct demonstration of the involvement of ET in the regulation of human cerebrovascular tone in physiological conditions.

The purpose of the present study was to assess whether endothelium-derived ET was involved in the overall responsiveness of freshly isolated human pial arteries by comparing responses to agonists after selective inhibition of various endothelium-derived factors with responses obtained after endothelial denudation of human pial arteries. The results reported in this manuscript demonstrate that ET may regulate...
human cerebrovascular tone during agonist stimulation and in conditions in which NO production is blocked.

Methods

Human pial arteries (382±21 μm, 69 rings), otherwise discarded, were obtained from 10 patients (who were 14, 16, 29, 32, 35, 37, 44, and 46 years of age; 8 males, 2 females) during neurosurgical resection of brain tumor (2 male patients) or epileptic lesion. None of the patients were diabetic or hypertensive, and none had coronary heart disease. Only normal arteries (those not feeding the tumor or included in the epileptic lesion) were used. They were transported in the laboratory in ice-cold PSS containing indomethacin (10 μmol/L, inhibitor of cyclooxygenase) and of the following composition (mmol/L): NaCl 130, KCl 4.7, KH2PO4 1.18, MgSO4 1.17, NaHCO3 14.9, EDTA 0.026, glucose 10, and aerated with 12% O2/5% CO2/83% N2 (pH 7.4). Segments of 2 mm long were mounted on 30 μm tungsten wires in resistance artery myograph (IMF) and either studied 1 hour, 8 hours, or 16 hours after surgery. No significant changes in reactivity were observed after 8 or 16 hours of surgery as reported by others. At least 6 segments from a patient were used. Two segments were used per protocol, one being a control. After a 1-hour stabilization period, arterial segments were challenged with a

Results

In all patients, acetylcholine (1 μmol/L) and substance P (0.1 μmol/L) induced relaxation of 32±9% and 82±5%, respectively, of segments preconstricted with 40 mmol/L KCl PSS (64±8% Emax). When arterial rings were preconstricted with angiotensin II (1 μmol/L; 19±7% Emax, n=3), acetylcho-
human pial arterial rings preconstricted with serotonin (Fig 1, lower trace); this response was endothelium dependent (Fig 2).

In the presence of L-NA, the relaxation induced by oxymetazoline was significantly decreased but not abolished (Fig 2).

Since BQ123 antagonized the preconstricting tone induced by serotonin (see above), it was not possible to obtain a concentration-dependent relaxation of arterial segments preconstricted with serotonin.

To investigate the possible involvement of EDHF, which would mediate α₂-adrenergic receptor–dependent relaxation by activating smooth muscle K⁺ conductance, we studied the effect of TEA (1 mmol/L), an inhibitor of Ca²⁺-activated K⁺ (KCa) channels, and glibenclamide (1 μmol/L), an inhibitor of ATP-sensitive K⁺ (KATP) channels, on vascular reactivity after inhibition of NO using L-NA. TEA and glibenclamide were added 20 minutes before testing the relaxant effect of oxymetazoline. In the presence of TEA or glibenclamide, the relaxation mediated by oxymetazoline was not modified (Fig 4).

Relaxations induced by pinacidil, an ATP-sensitive K⁺ channel agonist, represented 19±4%, 68±5%, and 92±6% of relaxation at 1, 3, and 10 μmol/L, respectively. In the presence of glibenclamide, relaxations were decreased to 0±0%, 0±0%, and 54±4% of relaxation at 1, 3, and 10 μmol/L, respectively (n=3 to 4, P<.05 versus in the absence of glibenclamide).

Discussion

The results of this study suggest first that ET is involved in the tonic response induced by NO synthase inhibition; second, part of the contractile response induced by serotonin is endothelium-dependent and sensitive to BQ123; and third, the data suggest that activation of α₂-adrenergic receptors generated an endothelium-dependent relaxation that was selectively inhibited by exogenous ET and insensitive to KCa and KATP channel inhibition.

Acetylcholine mediated endothelium-dependent relaxation of a similar amplitude as reported before and was sensitive to NO synthase inhibition. It is important to note that inhibition of NO production increased basal tone. In experimental conditions in which isometric myographs are used, arterial

Figure 1. Partial recording of two experiments performed on isolated segments of human pial artery. The top recording shows the inhibitory effect of BQ123 on the contractile response induced by a single concentration of serotonin (5-HT, 10 μmol/L). The lower trace shows the concentration-dependent relaxant effect of oxymetazoline (OXY, 0.01 to 30 μmol/L) of arterial segments preconstricted with serotonin.

Figure 2. Relaxation mediated by oxymetazoline (0.01 to 30 μmol/L) of human pial arterial segments preconstricted with serotonin (10 μmol/L) in control conditions (n=3), after endothelial removal (-Endo; n=3), or inhibition of NO production with L-NA (100 μmol/L; n=5). Results are expressed as mean±SEM. n represents the number of donors. *P<.05 versus control. ¶P<.05 versus L-NA.

Figure 3. Relaxation mediated by oxymetazoline (0.01 to 30 μmol/L) of human pial artery segments preconstricted with serotonin (10 μmol/L) after inhibition of NO production with L-NA (100 μmol/L; n=5) alone or combined with the addition of exogenous ET-1 (ET, 1 nmol/L; n=3). Results are expressed as mean±SEM. n represents the number of donors. ¶P<.05 versus L-NA.

Figure 4. Effect of TEA (1 mmol/L) and glibenclamide (GLI, 1 μmol/L) on the relaxation induced by oxymetazoline of human pial arteries preconstricted with serotonin (10 μmol/L; n=4 to 6 per group) in the presence of L-NA (1-NA, 100 μmol/L). n represents the number of donors.
segments do not develop myogenic tone by opposition to what is observed in isobaric conditions.\textsuperscript{34} This can be demonstrated by the addition of sodium nitroprusside or papaverine, which do not relax isometrically mounted vessels. Consequently, it is likely that any increase in tone observed in the presence of L-NA is induced by endothelium-derived constricting factors, since L-NA had no effect in denuded arteries. This hypothesis is further supported by the inhibitory effect of BQ123, suggesting that ET is actively involved in the constriction induced by L-NA. It would also confirm and give a functional correlate to previous studies showing that NO actively inhibits the release of stimulated endothelium-derived ET.\textsuperscript{35–38} However, it is possible that NO masks the constricting influence of ET in our experimental conditions without directly affecting ET production.\textsuperscript{39} In canine cerebral arteries, cyclooxygenase derivatives have been shown to be involved in both basal and agonist-stimulated tone in similar experimental conditions.\textsuperscript{18,29–41} Since all experiments were performed in the presence of indomethacin, by blocking the production of vasoactive cyclooxygenase products, we could not confirm that these previous findings are applicable to humans.

Thus, this first set of data suggests that basal tone of human pial arteries is actively regulated by the endothelium. Although we previously reported that both constricting and dilating endothelial factors were involved in the regulation of the overall vascular tone of the rat tail artery,\textsuperscript{42} this is the first time that ET is shown to be physiologically involved in the regulation of the human cerebrovascular tone.

In the presence of an intact endothelium, the contraction mediated by serotonin was reduced by BQ123, suggesting an endothelium-dependent component of the serotoninergic response that may involve ET. The concentration of BQ123 used in this study has been shown to antagonize angiotensin II–induced endothelium–dependent contraction of the isolated rat tail artery.\textsuperscript{43} As mentioned in the introduction, most stimuli regulate ET release at the level of gene transcription. However, our finding that serotonin has an immediate effect on ET release is not without precedent.\textsuperscript{35–38}

The importance of endothelium-derived ET in the net contractile response to serotonin is actively counteracted by NO. Inhibition of NO synthase significantly attenuated the relaxant properties of oxymetazoline (Fig 1). Activation of endothelial \(\alpha\)-adrenergic receptors induced NO release, triggering relaxation of large conductance arteries.\textsuperscript{45,46} In rat cerebral arteries, NO has been shown to have a permissive role on the relaxation induced by \(\alpha\)-adrenergic receptor agonists.\textsuperscript{47} However, as in our experimental conditions, NO appears not to be the only factor contributing to the \(\alpha\)-adrenergic receptor–mediated relaxation as previously reported by others.\textsuperscript{48}

Since the preconstricting tone is highly dependent on ET release, we hypothesized that oxymetazoline may cause relaxation of serotonin-preconstricted human pial arteries by decreasing ET production, counterbalancing the stimulatory effect of serotonin. It has been reported that oxymetazoline decreased ET production from cultured human pial artery ECs and isolated segments of rabbit middle cerebral artery.\textsuperscript{49} Since BQ123 abolished the contractile response to serotonin (Fig 1, Table 1), we were unable to construct relaxant concentration–response curves to oxymetazoline in these conditions. Thus, we postulated that if our hypothesis was valid, the addition of exogenous ET would not only potentiate the preconstricting tone but would also selectively antagonize the relaxation mediated by activation of \(\alpha\)-adrenergic receptors. Indeed, by adding ET we believed we would artificially inhibit the relaxant pathway, ie, the decrease in endothelial ET release by \(\alpha\)-adrenergic receptor occupation. As shown by Fig 3, the relaxation mediated by oxymetazoline was fully antagonized by ET, whereas substance P still induced a potent relaxation. Substance P has been shown to cause relaxation of human cerebral arteries by stimulating the release of both NO and EDHF\textsuperscript{50}; this would suggest that EDHF is not the mediator of the \(\alpha\)-adrenergic receptor–dependent pathway. Rather, a functional inhibition of ET release is likely, as previously reported in rabbit cerebral arteries and cultured human pial artery ECs.\textsuperscript{49}

This hypothesis is reinforced by the absence of effect of two well characterized inhibitors of potassium channels (Fig 4). TEA is an inhibitor of \(K_{\text{Ca}}\) channels\textsuperscript{52,48,49}; a TEA-sensitive pathway has been shown to be a key regulator of the mesenteric and brain circulations.\textsuperscript{19,30,50} In some vascular preparations, receptor-mediated endothelium–dependent smooth muscle cell relaxation could
be blocked by inhibitors of KCa channels but not KATP channels in the absence of NO production.325,33 The absence of effect of GLI is therefore not surprising.

In conclusion, our results reveal for the first time that ET may have a relevant physiological function in the human cerebral circulation in vitro. They show that NO actively counteracts ET production and/or action. They also suggest that receptor-activated ET regulation may play an important role in the overall control of the local cerebrovascular tone. It is noteworthy that in humans, serotonin has been proposed to be involved in the pathogenesis of migraine, and it has been suggested that treatment with ET receptor antagonists may be an efficient therapy for some forms of migraine.34–38 Although we acknowledge the possible limitations of this study due to the limited numbers of experiments performed in some protocols, altogether these studies suggest that there may be a link between ET, serotonin, and certain cerebrovascular disorders in humans.

References

Endothelial Regulation of Human Pial Arteries

Endothelial exerts a major influence on tone of underlying vascular muscle by production and release of potent relaxing and contracting factors. The endothelium-derived contracting factor that has received the most attention is endothelin, a peptide originally isolated from porcine arterial endothelium. Vascular effects of endothelin are mediated through activation of two receptors, endothelin-A and endothelin-B receptors. In general, endothelin-A receptors are expressed in vascular muscle and mediate contraction to endothelin. In contrast, endothelin-B receptors are expressed on endothelium and can mediate endothelium-dependent relaxation. Although these concepts were based initially on findings made with use of vessels from animal models, the same mechanisms have been described in cerebral arteries from humans.

The results of the present study are interesting because they suggest an additional effect of endothelin. Based on data obtained with use of human pial arteries, the findings suggest that endothelin may selectively modulate responses to other vasoactive stimuli. For example, relatively low concentrations of endothelin impaired endothelium-dependent relaxation to acetylcholine in rat isolated mesenteric artery. Based on data from animal models, the same mechanisms have been described in cerebral arteries from humans. The results of the present study are interesting because they suggest an additional effect of endothelin. Based on data obtained with use of human pial arteries, the findings suggest that endothelin may selectively modulate responses to other vasoactive stimuli. For example, relatively low concentrations of endothelin impaired endothelium-dependent relaxation to acetylcholine in rat isolated mesenteric artery. Based on data from animal models, the same mechanisms have been described in cerebral arteries from humans.

An additional interesting aspect of the present study relates to potential interaction of endothelin with nitric oxide. Previous studies suggest that in addition to being a powerful vasodilator, nitric oxide also inhibits gene expression and/or synthesis of endothelin. In the present study, pharmacological inhibition of nitric oxide synthase appeared to unmask a constrictor effect of endogenous endothelin. An implication of this finding is that endothelin may exert a greater influence on vascular tone under pathological conditions which are associated with impairment of the nitric oxide signaling pathway.

**Frank M. Faraci, PhD, Guest Editor**
Department of Internal Medicine
Cardiovascular Division
University of Iowa College of Medicine
Iowa City, Iowa

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