RESEARCH PAPER

Dominance of flow-mediated constriction over flow-mediated dilatation in the rat carotid artery

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BACKGROUND AND PURPOSE

The shearing forces generated by flow generally evoke dilatation in systemic vessels but constriction in the cerebral circulation. The aim of this study was to determine the effects of flow on the conduit artery delivering blood to the brain in the rat, that is, the carotid artery.

EXPERIMENTAL APPROACH

Carotid artery segments were mounted in a pressure myograph and pressurized to 100 mmHg. Changes in vessel diameter to flow (0.5–10 mL·min^{-1} for 2–10 min) at constant pressure were then measured using a video dimension analyser.

KEY RESULTS

Following the induction of tone, the onset of flow evoked a transient dilatation followed by a powerful constriction that was sustained until the termination of flow. Endothelial denudation or treatment with indomethacin, N^G-nitro-L-arginine methyl ester, or the combination of apamin and TRAM–34 showed that the initial flow-mediated dilatation arose from the combined actions of endothelium-derived NO and endothelium-derived hyperpolarizing factor (EDHF). The flow-mediated constriction, which increased in magnitude with increasing flow rate and duration of flow, was also endothelium dependent, but was unaffected by treatment with superoxide dismutase, BQ-123, indomethacin, HET0016 or carbenoxolone. Flow-mediated constriction therefore appeared not to involve superoxide anion, endothelin-1, a COX product, 20-HETE or gap-junctional communication.

CONCLUSIONS AND IMPLICATIONS

Although a weak, transient flow-mediated dilatation is observed in the rat carotid artery, the dominant response to flow is a powerful and sustained constriction. Whether this flow-mediated constriction in the carotid artery serves as an extracranial mechanism to regulate cerebral blood flow remains to be determined.

Abbreviations

BQ-123, cyclo[D-Trp-D-Asp-Pro-D-Val-Leu]; DMSO, dimethylsulfoxide; EDHF, endothelium-derived hyperpolarizing factor; HET0016, N-(4-butyl-2-methylphenyl)-N'-hydroxy-methanimidamide; L-NAME, N^G-nitro-L-arginine methyl ester; SOD, superoxide dismutase; TRAM-34, 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole; U46619, 9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin F$_{2\alpha}$

Introduction

Endothelial cells in the vascular wall are subjected to a variety of distinct haemodynamic forces, including the longitudinal shearing force of the flowing blood and the radial cyclic strain induced by the pulsatile changes in blood pressure occurring during the cardiac cycle (Busse and Fleming, 2003). These forces have a profound influence on

endothelial phenotype through the regulated expression of multiple genes controlling anti-atherogenic, anti-inflammatory, anti-apoptotic and anti-proliferative functions (Bongrazio *et al.*, 2000; Chen *et al.*, 2001; Wasserman *et al.*, 2002; Lamack and Friedman, 2007). In addition, these forces have a more immediate function in regulating vascular tone and hence, blood flow and pressure. In the majority of systemic arteries and arterial beds, shearing forces acting on the luminal surface elicit flow-mediated dilatation, resulting from the release and actions of either one or a combination of the endothelium-derived vasodilators, NO, endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (Sun *et al.*, 1999; Dube and Canty, 2001; Huang *et al.*, 2001; Fujioka *et al.*, 2004; Bellien *et al.*, 2006). Although flow-mediated dilatation has also been observed in rat and rabbit cerebral arteries (Fujii *et al.*, 1991; Gaw and Bevan, 1993), reports of flow-mediated constriction appear to predominate in rat and cat cerebral arteries (Madden and Christman, 1999; Bryan *et al.*, 2001a,b; New *et al.*, 2003). In pig cerebral arteries, both flow-mediated dilatation and constriction have been reported, depending on flow rate (Shimoda *et al.*, 1996). Flow-mediated constriction has also been observed in pig and cat small pulmonary arteries (Shimoda *et al.*, 1997; Liu *et al.*, 1998) and in the rabbit femoral artery (Hoogerwerf *et al.*, 1992; Clifford *et al.*, 2010). Furthermore, flow-mediated dilatation in rat gracilis muscle arterioles and venules is reversed to constriction following the development of hyperhomocysteinemia (Bagi *et al.*, 2002; Racz *et al.*, 2010).

The cerebral circulation is somewhat unusual in that large proximal arteries, such as the middle cerebral artery, are important determinants of vascular resistance, rather than the small arteries typical of systemic vascular beds (Bohlen, 1987; Faraci and Heistad, 1990; 1998). Cerebral microvascular pressure is therefore greatly influenced by the tone of large feeder arteries, and flow-mediated constriction of these has been regarded as a mechanism that protects small, thin-walled intracranial vessels from high pressures that might otherwise provoke haemorrhage (Faraci and Heistad, 1998; New *et al.*, 2003).

As the carotid arteries are the main conduits to the brain, we wished to determine if these too might play a role in cerebrovascular protection by exhibiting flow-mediated constriction. Indeed, in this study we showed that while rat carotid artery exhibits a weak, flow-mediated dilator response, this is dominated by a much more powerful and sustained flow-mediated constriction.

Methods

Flow-mediated vasodilatation and constriction in the rat carotid artery assessed by pressure myography

All animal care and experimental procedures complied with the UK Home Office regulations. Female Wistar rats (83 in total) weighing 150–200 g were killed by an overdose of CO_2. The carotid arteries were removed, cleared of adhering fat and connective tissue, and trimmed to 1.5 cm long segments. All the studies involving animals are reported in accordance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Flow-mediated dilatation and constriction in the carotid artery was measured at constant pressure using a pressure myograph equipped with a video dimension analyser (Danish Myo Technology, model 11OP). The video dimension analyser measured only outer diameter because the vessels were not transparent. The carotid artery segments were cannulated at both ends and mounted in the heated (37°C) microscope stage bath (volume 10 mL) filled with Krebs solution comprising (mM): NaCl 118, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 24, glucose 11, and gassed with 95% O_2 and 5% CO_2. The vessels were then pressurized to an inflow pressure of 100 mmHg by infusion with oxygenated Krebs delivered via the inflow cannula and by raising the height of the end of a 2 m long, 3 mm internal diameter, silicone rubber outflow tube held on a rigid scaffold. Vessels were allowed to equilibrate in the absence of any flow for 1 h before experiments were begun. Flow-mediated dilator and constrictor responses were elicited by inducing flow using a peristaltic pump (Gilson Minipuls model 3). Periods of flow ranged from 2 to 10 min at flow rates of 0.5–10 mL·min⁻¹. Except where explicitly stated, pressure was held constant at 100 mmHg by lowering the height of the outflow tubing during flow and raising it again at the end of flow; it took ~15 s to stabilize pressure when flow was initiated or stopped. Where indicated, some experiments were also conducted at 25, 50, 75 and 125 mmHg. A pulse dampener was employed to reduce the pulse pressure fluctuations caused by the peristaltic pump and ensure flow was laminar; this was placed in the inflow circuit between the pump and myograph and consisted of a sealed glass chamber (10 mL volume) with an inflow tube at the bottom and outflow at the 5 mL mark, giving a cushioning volume of air above the perfusion fluid. This also acted as a bubble trap, preventing bubbles of gas reaching the tissue.

In some experiments, the endothelium was removed by pinning the carotid artery segments at both ends on a silicone resin-filled Petri dish and gently inserting into the lumen a 500 μm diameter stainless steel wire five times. Pinning ensured that arterial segments were not subjected to longitudinal stretch when the luminal surface was being abraded. Endothelial denudation was deemed to have been successful by the absence of flow-mediated dilatation.

Experimental protocols

As will be seen in the Results section, induction of flow had virtually no effect on vessel diameter in pressurized carotid artery segments at resting levels of tone. Preliminary experiments demonstrated, however, that a constant low level of tone (~50 μm constriction) induced using the thromboxane-mimetic U46619 (5–20 nM) provided ideal conditions for examining flow-mediated constriction; raising tone to an intermediate level (~100 μm constriction) using 5–30 nM U46619 permitted examination of biphasic flow-mediated responses, consisting of an initial dilatation followed by constriction; and raising tone yet further to ~75% of the maximum achievable (~300 μm constriction) using 30–150 nM U46619 permitted examination of flow-mediated dilatation against a stable baseline without flow-mediated constriction obscuring the response.

In some experiments, superoxide dismutase (SOD, 100 u·mL⁻¹), catalase (1200 u·mL⁻¹), BQ-123 (1 µM), indomethacin (3 µM), HET0016 (3 µM) and carbenoxolone (100 µM) were used to determine, respectively, the potential involvement of superoxide anion, hydrogen peroxide, endothelin, COX products, 20-HETE or gap-junctional communication in the flow-mediated constrictor response. In these and all other experiments, the drugs were added to both the bath and perfusion fluid for a minimum of 1 h to ensure adequate diffusion into the vessel. A further set of experiments was conducted to determine the role of calcium ions in the flow-mediated constrictor response. In these, the standard Krebs solution was replaced with one lacking calcium, but containing the calcium chelator, EGTA, 1 mM.

In other experiments, N^G-nitro-L-arginine methyl ester (L-NAME; 100 µM), indomethacin (3 µM) or the combination of apamin (0.5 µM) and TRAM-34 (10 µM) were used to determine, respectively, the potential involvement of NO, COX products and EDHF in the flow-mediated dilator response. In yet other experiments, all four of these blocking agents were combined to determine if inhibition of the endothelium-derived vasodilator agents affected the magnitude of flow-mediated constriction.

At the end of every experiment, papaverine (300 µM) was added to dilate fully the vessels and enable calibration of flow-mediated dilator responses.

All drug and molecular target nomenclature conforms to the Br. J. Pharmacol. Guide to Receptors and Channels (Alexander *et al.*, 2011).

Drugs and chemicals

Apamin, indomethacin, L-NAME, papaverine hydrochloride, SOD (from bovine erythrocytes), TRAM-34 and U46619 were all obtained from Sigma, Poole, UK. Catalase (bovine liver) was obtained from Calbiochem UK and BQ-123 and carbenoxolone disodium from Tocris UK. HET0016 was obtained from Cayman Chemical, Ann Arbor, MI, USA. All drugs were dissolved in 0.9% saline, with the following exceptions: BQ-123 (1 mM stock) in distilled water; indomethacin (10 mM stock) in Na_2CO_3 (3 mM); TRAM-34 (10 mM stock) and HET0016 (10 mM stock) in dimethylsulfoxide (DMSO); and U46619 (1 mM stock) in 50% ethanol. Control experiments showed that at the concentrations employed, these solvents themselves had no effect on the carotid artery.

Data analysis

Changes in vessel outer diameter, reflecting flow-mediated constrictor and dilator responses, were measured in µm, although in some cases the latter were converted to a percentage of the maximum dilatation achieved by papaverine (300 µM), which was added at the end of each experiment. Data are expressed as the mean ± SEM of n separate observations, each from a separate tissue. Graphs were drawn and statistical comparisons were made using Student's t-test or one-way ANOVA and Bonferroni's post-test, as appropriate, with the aid of a computer programme, Prism (GraphPad, San Diego, CA, USA). A probability (P) less than or equal to 0.05 was considered significant.

Results

Effects of tone on flow-mediated responses

Following pressurization at 100 mmHg, rat carotid artery segments had a resting outer diameter of 1081 ± 9 µm (n = 33). When papaverine (300 µM) was added at the end of each experiment to produce complete relaxation, the diameter was not significantly different (1100 ± 9 µm), suggesting a low level of myogenic tone (19 ± 4 µm constriction).

In the absence of any vasoconstrictor agent, the induction of flow at 5 mL·min⁻¹ for 5 min at constant pressure elicited a barely perceptible constriction of 10 ± 4 µm (n = 5; Figure 1). The sequential addition of L-NAME (100 µM), indomethacin (3 µM), then apamin (0.5 µM) and TRAM-34 (10 µM), to block any opposing dilator effects of NO, prostanoids or EDHF, had no effect on the magnitude of this constriction. In the continued presence of these blocking agents, and following the induction of ~10% maximal tone (48 ± 5 µm constriction) using U46619 (5–20 nM), flow elicited a small transient dilatation followed by a more powerful, slowly developing constriction (87 ± 2 µm) that was sustained until the termination of flow. While this basal level of tone was necessary to uncover flow-mediated constriction, the presence of the blocking agents was not; in their absence flow (5 mL·min⁻¹ for 5 min) elicited a similar constrictor response (102 ± 9 µm, n = 13). The use of phenylephrine to induce a low level of tone similarly uncovered the ability of flow to induce constriction (data not shown).

Effects of SOD, catalase, BQ-123, indomethacin, HET0016, carbenoxolone, Ca^{2+}-free solution, endothelial denudation and distending pressure on flow-mediated constriction

In the presence of ~10% maximal tone (58 ± 17 µm; n = 8) induced using U46619 (10–50 nM), flow at 5 mL·min⁻¹ for 5 min elicited robust constriction of 91 ± 6 µm (Figure 2). This flow-mediated constriction was unaffected by SOD (100 u·mL⁻¹), either alone or in combination with catalase (1200 u·mL⁻¹), by the ET_A-receptor blocker BQ-123 (1 µM), indomethacin (3 µM), HET0016 (3 µM) or carbenoxolone (100 µM). Conducting experiments in Ca^{2+}-free solution (containing EGTA, 1 mM) or following endothelial denudation did, however, significantly impair flow-mediated constriction.

While originally set at ~10% maximal U46619-induced tone (at 100 mmHg), the effects of flow at 5 mL·min⁻¹ for 5 min were examined over a range of distending pressures (25–125 mmHg). Vessel diameter increased with increasing pressure throughout this range (Figure 3). The magnitude of the flow-mediated constriction was small at 25 and 50 mmHg, rose steeply at 75 mmHg, was optimal at 100 mmHg and fell back at 125 mmHg.

Effects of varying flow rate and duration of flow on flow-mediated responses

In the absence of any blocking agents, and at an intermediate level of tone (105 ± 23 µm constriction) induced using
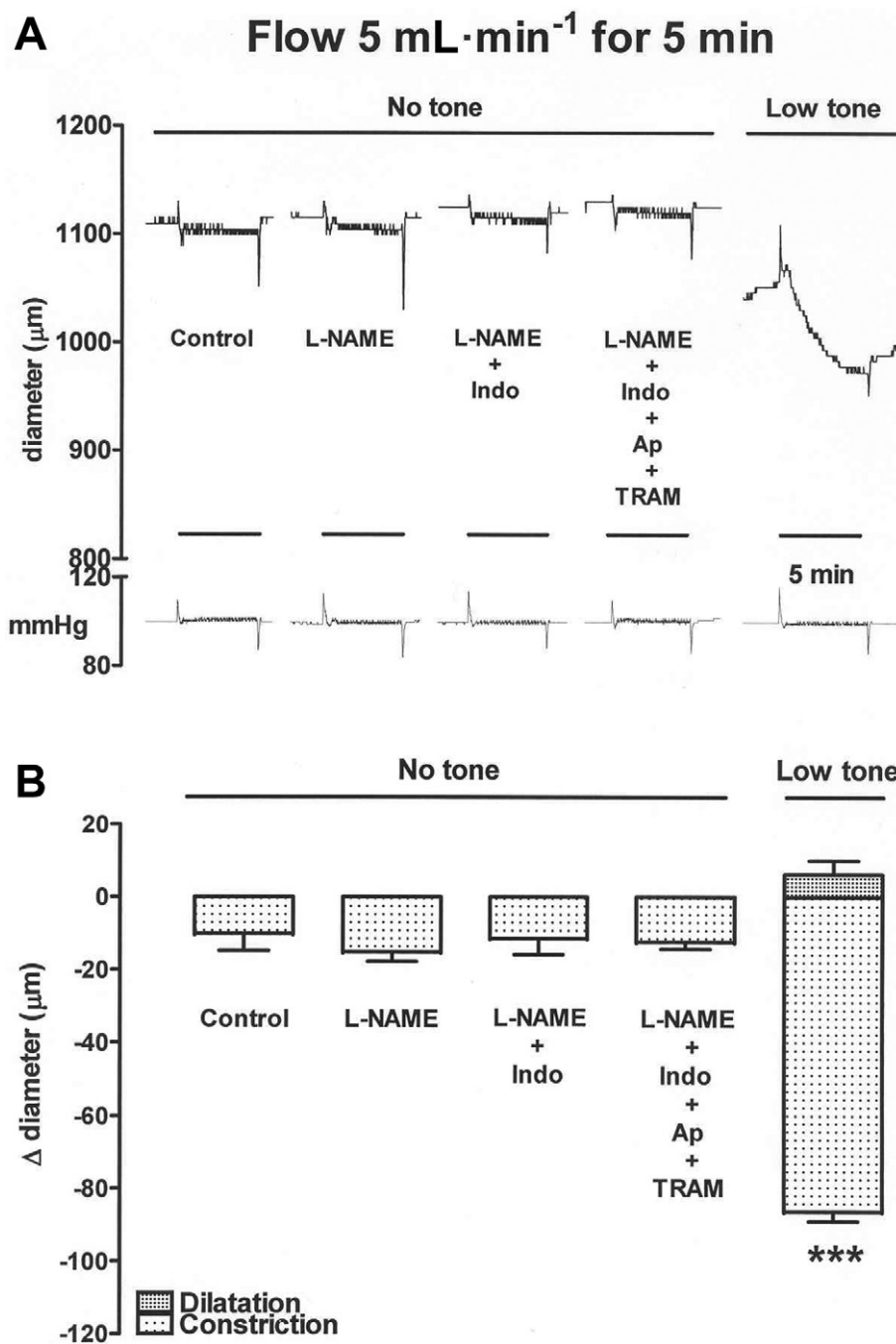
Figure 1

An experimental trace (A) and the results from several experiments (B) showing that in the absence of tone, flow for 5 min at 5 mL·min^{-1} elicited only a barely detectable constrictor response in rat carotid artery segments. The sequential addition of L-NAME (100 µM), indomethacin (Indo, 3 µM), then apamin (Ap, 0.5 µM) and TRAM-34 (10 µM), to block any opposing dilator effects of NO, prostanoids or EDHF, had no effect on the magnitude of this constriction. However, following induction of low-level tone using U46619, flow elicited a small transient dilatation followed by a more powerful constriction that was sustained until the termination of flow. The 5 min periods of flow are indicated by the time bars. The pressure trace (in mmHg) shows that flow was induced while maintaining constant pressure. Data are presented as the mean ± SEM of five observations. ***$P < 0.001$ indicates a significant difference from control.

U46619 (5–30 nM), low rates of flow elicited a biphasic response, comprising an initial dilatation that was interrupted by the onset of a more slowly developing constriction that was sustained until the termination of flow; higher rates of flow elicited mainly constriction (Figure 4). Figure 5 gives fuller details of how the magnitude of these dilatations and constrictions varied with flow rate (0.5–10 mL·min^{-1}) and duration of flow (2–10 min).

Flow 5 mL·min⁻¹ for 5 min
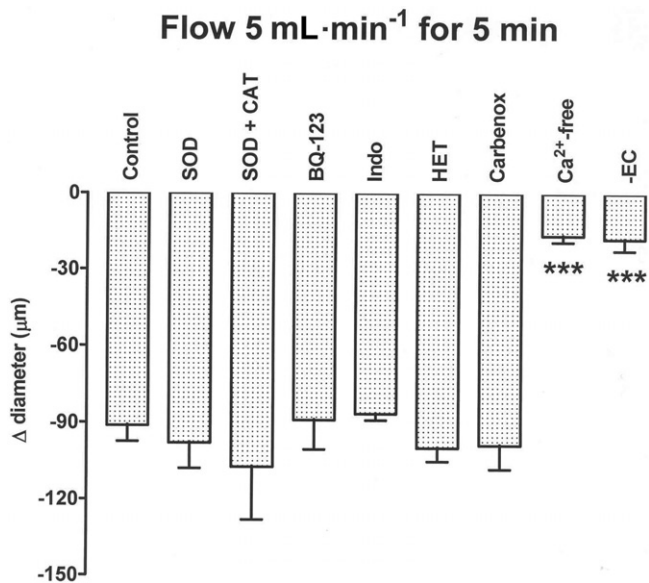
Figure 2

Bar graph showing constriction induced by flow at 5 mL·min⁻¹ for 5 min in carotid artery segments at a low level of U46619-induced tone. This flow-induced constriction was unaffected by superoxide dismutase (SOD, 100 u·mL⁻¹), either alone or in combination with catalase (CAT, 1200 u·mL⁻¹), by BQ-123 (1 μM) alone, by indomethacin (Indo, 3 μM) alone, by HET0016 (HET, 3 μM) alone, or by carbenoxolone (Carbenox, 100 μM) alone, but was powerfully inhibited in Ca^{2+}-free solution, or by endothelial denudation (-EC). Data are presented as the mean ± SEM of 4–10 observations. ***$P < 0.001$ indicates a significant difference from control.

Flow 5 mL·min⁻¹ for 5 min
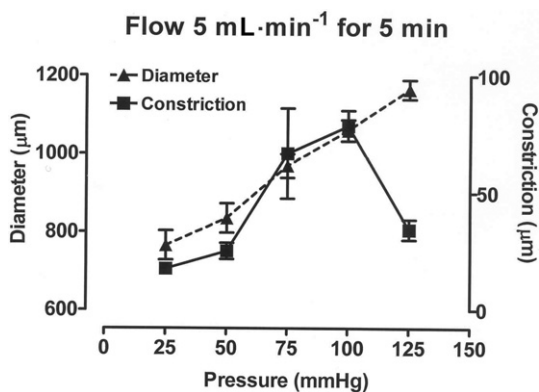
Figure 3

Graph showing how the external diameter and the constriction induced by flow at 5 mL·min⁻¹ for 5 min varied over a range of distending pressures (25–125 mmHg) in carotid artery segments. All vessels were initially set at a low level of U46619-induced tone while at a pressure of 100 mmHg. Data are presented at the mean ± SEM of 5 observations.

When the duration of flow was 2 min, the magnitude of the dilatation increased with increasing flow rate up to a maximum of 34 ± 6 μm ($n = 11$) at 2.5 mL·min⁻¹ and declined thereafter (Figure 5); at the optimum flow rate, the latency for

A Flow rate (mL·min⁻¹ for 10 min)

B
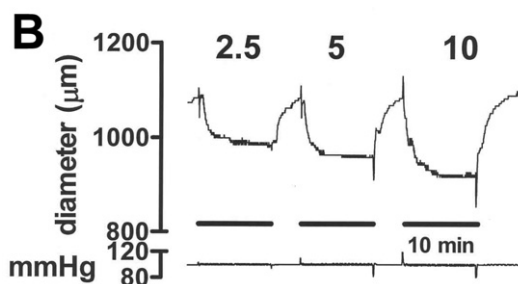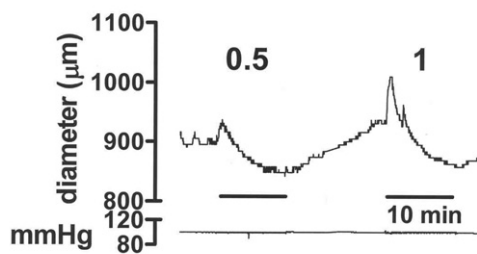
Figure 4

Experimental traces showing the effects of inducing flow for 10 min periods at (A) 0.5 and 1.0 mL·min⁻¹ and (B) 2.5, 5.0 and 10 mL·min⁻¹ in carotid artery segments at an intermediate level of U46619-induced tone. Traces (A) and (B) were from different vessels. Low rates of flow induced a biphasic response, comprising an initial dilatation, followed by a more slowly developing constriction that was sustained until the termination of flow. Higher rates of flow induced mostly constriction. The 10 min periods of flow are indicated by the time bars. The numbers on the trace indicate the flow rate in mL·min⁻¹. The pressure traces (in mmHg) show that flow was induced while maintaining constant pressure.

onset of dilatation and the time to peak were 17 ± 2 s and 32 ± 2 s, respectively. With flow for longer periods (5 and 10 min), the maximum dilatation occurred at 1 mL·min⁻¹. No dilatation was seen at a flow rate of 10 mL·min⁻¹, regardless of the duration of flow.

Constriction was clearly the dominant response of the tissue to flow, and its magnitude increased with increasing flow rate and duration of flow, reaching 176 ± 25 μm ($n = 5$) at 10 mL·min⁻¹ for 10 min (Figure 5); at this flow rate, the latency for onset of constriction and the time to peak were 16 ± 2 s and 407 ± 96 s, respectively.

In the combined presence of L-NAME (100 μM), indomethacin (3 μM), apamin (0.5 μM) and TRAM-34 (10 μM), the initial dilatation induced by flow (0.5–5 mL·min⁻¹ for 2 min) was inhibited, but the magnitude of the ensuing constriction was unaffected (Figure 6).

Effects of L-NAME, indomethacin, apamin and TRAM-34, and endothelial denudation on flow-mediated dilatation

In the presence of ~75% maximal tone (289 ± 27 μm constriction; $n = 5$) induced using U46619 (30–150 nM), flow-mediated dilatation could be observed against a stable baseline without flow-mediated constriction obscuring the

A
Flow 2 min

B
Flow 5 min

C
Flow 10 min
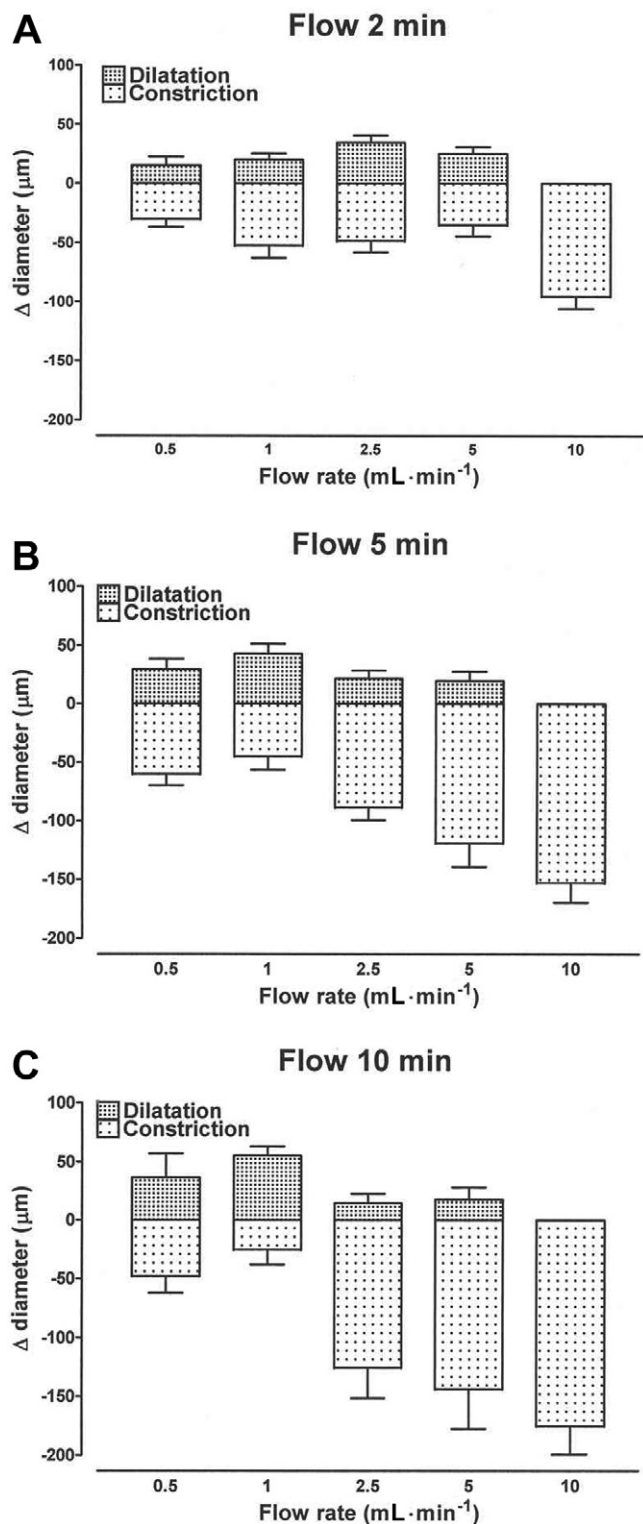
Figure 5

Bar graphs showing how flow-induced dilatation and constriction varied with flow rate (0.5–10 mL·min^{-1}) and with the duration of flow for 2 min (A), 5 min (B) and 10 min (C) in carotid artery segments at an intermediate level of U46619-induced tone. Data are presented as the mean ± SEM of 5–11 observations.

Flow 2 min
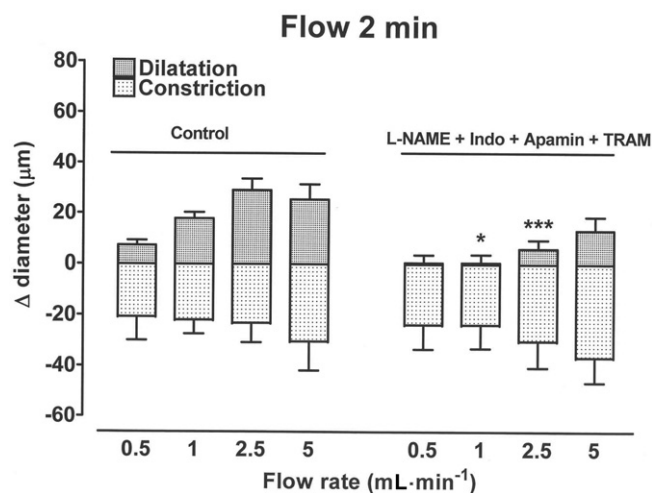
Figure 6

Bar graph showing how flow for 2 min periods at a range of flow rates (0.5–5 mL·min^{-1}) induced dilatation and constriction in carotid artery segments at an intermediate level of U46619-induced tone. The combined presence of L-NAME (100 µM), indomethacin (Indo, 3 µM), apamin (0.5 µM) and TRAM-34 (10 µM) inhibited the dilatation but had no effect on the magnitude of the flow-induced constriction. Data are presented as the mean ± SEM of five to nine observations. *$P < 0.05$ and ***$P < 0.01$ indicate significant differences from respective controls.

response (Figure 7A). Under these conditions, induction of flow at 0.5–5 mL·min^{-1} for 2 min elicited graded relaxations that peaked at 2.5 mL·min^{-1} (Figure 7A,B). The COX blocker, indomethacin (3 µM), had no effect by itself on flow-mediated dilatation (data not shown) and neither did its combination with the NO synthase blocker, L-NAME (100 µM; Figure 7B). The EDHF blockers, apamin (0.5 µM) and TRAM-34 (10 µM), when combined with indomethacin, did inhibit flow-mediated dilatation, and the magnitude of this blockade was enhanced further by L-NAME. Like control tissues, endothelium-denuded segments of carotid artery constricted in response to U46619, but they did not dilate in response to flow (data not shown).

Discussion

The major new finding presented in this study is that while a weak, transient flow-mediated dilatation can be seen in the rat carotid artery, the dominant response of the vessel to flow at a constant pressure of 100 mmHg is a profound and sustained vasoconstriction. The magnitude of the constrictor response is proportional to the rate of flow and the response is reversible, decaying rapidly upon cessation of flow.

In most systemic arteries and arterial beds, the shearing forces generated by flow elicit vasodilatation, which is uniformly found to be endothelium-dependent, and results from the release of either one or a combination of the endothelium-derived vasodilators, NO, EDHF or prostacyclin (Sun *et al.*, 1999; Dube and Canty, 2001; Huang *et al.*, 2001; Fujioka *et al.*, 2004; Bellien *et al.*, 2006). These dilator
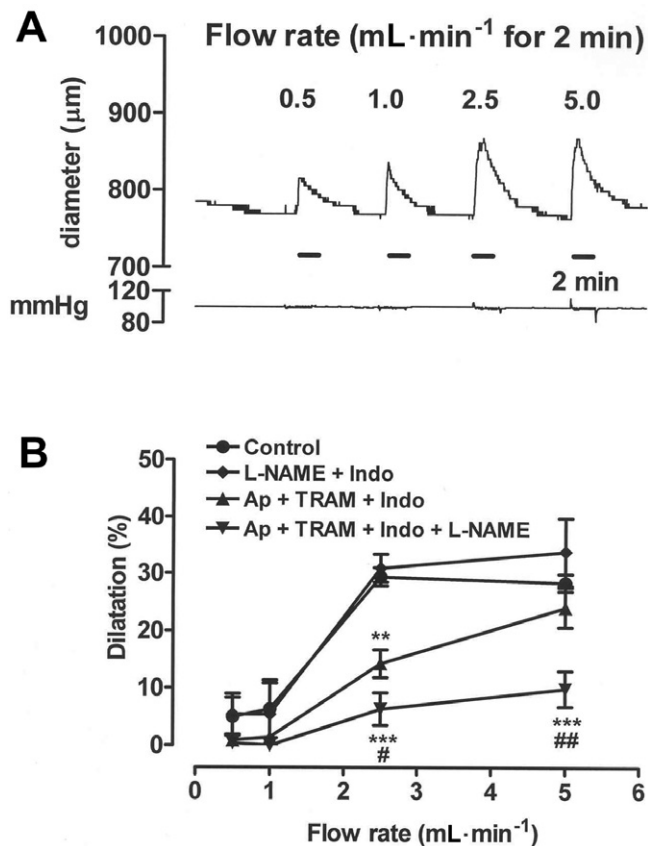
Figure 7

An experimental trace (A) and the results from several experiments (B) showing how flow-induced dilatation varied with flow rate (0.5–5 mL·min^{-1} for 2 min) in carotid artery segments at ~75% of maximal U46619-induced tone. Dilatation was unaffected by combined treatment with L-NAME (100 μM) and indomethacin (Indo, 3 μM), but was inhibited by the combination of apamin (Ap, 0.5 μM) and TRAM-34 (10 μM) with indomethacin. Inhibition by the latter combination was enhanced following additional inclusion of L-NAME. The 2 min periods of flow are indicated by the time bars. The pressure trace (in mmHg) shows that flow was induced while maintaining constant pressure. Data are presented as the mean ± SEM of four or five experiments. **$P < 0.01$ and ***$P < 0.001$ indicate significant differences from respective controls. #$P < 0.05$ and ##$P < 0.01$ indicate a significant difference from tissues treated with the combination of apamin, TRAM-34 and indomethacin.

responses are explained by the shearing forces acting on the endothelium, elevating the level of intracellular calcium (Hoyer *et al.*, 1998), which promotes the activation of the calcium-dependent potassium channels necessary for the action of EDHF (Busse *et al.*, 2002), calcium-sensitive phospholipase A$_2$ needed for the synthesis of prostacyclin (Chang *et al.*, 1987), and calcium-calmodulin-sensitive NO synthase (Salter *et al.*, 1991). Sustained release of NO by flow, however, occurs through the continuous activation of the phosphatidylinositol 3-kinase and subsequent kinase PKB (Akt) and PKA pathways, which activate endothelial NO synthase by phosphorylation at Ser[1177] (Dimmeler *et al.*, 1999). In the rat carotid artery examined in this study, the initiation of flow (0.5–5 mL·min^{-1}) at constant pressure elicited dilator

responses, but these were transient, peaking at around 30 s, and their magnitude was almost certainly curtailed by the onset of the more slowly developing but sustained constrictor response. The dilatations were best observed using short (2 min) durations of flow, which reduced the magnitude of the opposing constrictor response, and at relatively high levels of vessel tone (~75% of max induced by U46619), which helped maintain a stable baseline. Under these conditions, the magnitude of the dilator response increased with increasing flow rate over the range 0.5–2.5 mL·min^{-1} and declined at higher rates of flow. At 10 mL·min^{-1} no dilatation was seen, presumably because the magnitude of the concomitant flow-mediated constriction was so intense. In keeping with previous reports, flow-mediated dilatation was abolished following endothelial denudation (Sun *et al.*, 1999; Dube and Canty, 2001; Fujioka *et al.*, 2004). Unlike in rat and mouse skeletal muscle arterioles (Koller *et al.*, 1994; Sun *et al.*, 1999), the COX product, prostacyclin, appeared to play no role in the flow-mediated dilator response in the rat carotid artery, because indomethacin was without effect. Although the NO synthase blocker, L-NAME, had no effect by itself on flow-mediated dilatation, it did enhance the blockade induced by the combination of apamin and TRAM-34 (Waldron and Garland, 1994; Crane *et al.*, 2003). It is therefore likely that flow-mediated dilatation in the rat carotid artery is the result of the combined effects of EDHF and NO, as is also the case in the human radial artery and coronary arterioles (Miura *et al.*, 2001; Bellien *et al.*, 2006). We acknowledge that our use of the term 'EDHF' is far from ideal because it can equally apply to situations where dilatation occurs by electrotonic spread of hyperpolarization and thus not through the action of a chemical mediator *per se*, or when it is not blocked by the combination of apamin and TRAM-34, such as when it is mediated by epoxyeicosatrienoic acids. It is therefore appropriate to use this term with caution when the precise mechanisms underlying vasodilatation are unknown (Busse *et al.*, 2002; Félétou and Vanhoutte, 2006).

Although the onset of the flow-mediated constrictor response clearly suppresses the flow-mediated dilatation, blocking the dilatation with the combination of L-NAME, indomethacin, apamin and TRAM-34 has no effect on the magnitude of the constriction. It is therefore likely that the magnitude of the flow-mediated dilatation is too weak to produce effective physiological antagonism of the more powerful concomitant constriction. Although the majority of experiments were conducted at a constant pressure of 100 mmHg, the magnitude of the flow-induced constriction was seen to increase with increasing pressure from 25 to 100 mmHg and declined at 125 mmHg. The magnitude of the flow-mediated constriction was also related to the flow rate (0.5–10 mL·min^{-1}) and to the duration of flow (2–10 min), and was thus proportional to the shear stress acting on the vessel wall. Shear stress is inversely related to the third power of the internal radius (Sun *et al.*, 1999), and this probably explains why flow-mediated constriction was absent at basal tone but was uncovered following low-level constriction with either U46619 or phenylephrine. Flow-mediated constriction has previously been found in a number of other arteries and arterial beds, but its precise origins are still a matter of debate. For example, in cat and pig pulmonary arteries and piglet cerebral arteries (Shimoda *et al.*, 1996;

1997; Liu *et al.*, 1998), flow-mediated constriction is reported to be endothelium dependent, whereas in rat and cat cerebral arteries (Madden and Christman, 1999; Bryan *et al.*, 2001b; New *et al.*, 2003) it appears to be independent of the endothelium. Why different vascular sites appear to differ so radically in this respect is presently unknown, but in the rat carotid artery flow-mediated constriction was virtually abolished by endothelial denudation, suggesting it occurs by activation of an endothelium-derived process.

At vascular sites where the process of flow-mediated constriction is endothelium dependent, it could occur by a variety of mechanisms, for example, by electrotonic spread of a signal through myoendothelial gap junctions, or by release from the endothelium of either a vasoconstrictor agent or, alternatively, a substance that removes the influence of a tonically active vasodilator. Although inhibitors of gap-junctional communication, such as carbenoxolone, are known to impair the vasodilatation induced by the putative EDHF (Chaytor *et al.*, 2000; Kenny *et al.*, 2002), there are no reports of this agent inhibiting flow-mediated constriction. In fact, we found that carbenoxolone had no effect on flow-mediated constriction in the rat carotid artery, ruling out involvement of gap-junctional communication. In pig pulmonary arteries and rat skeletal muscle arterioles (Liu *et al.*, 1998; Bagi *et al.*, 2002), flow-induced constriction is impaired by treatment either with a NO synthase inhibitor or with SOD, either alone or in combination with catalase, suggesting it occurs indirectly through the production of superoxide anion, which destroys NO thereby removing a background vasodilator activity. This is clearly not the mechanism by which flow elicits constriction in the rat carotid artery, because we found that neither L-NAME nor SOD, either alone or with catalase, has any effect in this tissue. It is therefore more likely that flow elicits release of a directly acting vasoconstrictor from the endothelial cells in the rat carotid artery. Three potential candidates that seemed worthy of consideration are endothelin-1, thromboxane A_2, and 20-HETE, as flow-mediated constriction is inhibited by the ET_A-receptor blocker, BQ-123, in the cat pulmonary artery (Shimoda *et al.*, 1997), by the TP receptor or COX blockers, SQ 29,548 and indomethacin, respectively, in rat skeletal muscle venules and arterioles (Bagi *et al.*, 2002; Racz *et al.*, 2010), and by SQ 29,548, indomethacin, or the 20-HETE synthesis inhibitor, HET0016, in rat and human cerebral arteries (Toth *et al.*, 2011). In the rat carotid artery, however, we found that BQ-123, indomethacin and HET0016 each had no effect on flow-mediated constriction, ruling out the involvement of endothelin-1, a COX product or 20-HETE. Thus, in rat carotid artery, flow-mediated constriction appears to involve the release of an as yet unidentified endothelium-derived mediator. Experiments in Ca^{2+}-free solution suggest, however, that Ca^{2+} ions are involved in the release of this agent.

Blood flow through the carotid artery in anaesthetized rats *in vivo* varies with age (Kohler and Jawien, 1992; Miyashiro *et al.*, 1997), and is around 2.6 mL·min^{-1} for the 150–200 g rats used in our study. As this falls within the rates of flow $0.5–10 \text{ mL·min}^{-1}$ we used in our experiments, it is possible that flow-mediated constriction may be of physiological importance in the rat carotid artery, and could potentially function to limit cerebral blood flow. This is suggested because in the cerebral circulation, large proximal arteries such as the middle cerebral artery are important determinants of vascular resistance (Bohlen, 1987; Faraci and Heistad, 1990; 1998). As a consequence, it has been proposed that flow-mediated constriction in these large feeder arteries may protect the more delicate, thin-walled intracranial vessels from high pressures that could provoke haemorrhagic stroke (Faraci and Heistad, 1998; New *et al.*, 2003). Thus, flow-mediated constriction in the rat carotid artery could, by limiting cerebral blood flow, function as an extension of this intracranial protective mechanism.

The myogenic response, by which increases in transmural pressure elicit increases in vascular tone (Bayliss, 1902), is an important characteristic of cerebral vessels (Harder, 1984; Harder *et al.*, 2011), and acts in concert with the flow-mediated constrictor responses discussed above to hold brain blood flow constant over a wide range of pressures. We found, however, that the rat carotid artery displays very little myogenic tone, as revealed by the smooth muscle relaxant, papaverine, even when pressurized to 100 mmHg. Therefore, this important component of the autoregulatory function of cerebral arteries is absent in the carotid artery.

In conclusion, while a weak, transient flow-mediated dilatation resulting from the combined actions of NO and EDHF occurs in the rat carotid artery, the dominant response of the vessel to flow is a profound and sustained vasoconstriction. This flow-mediated vasoconstriction is endothelium-dependent, but is not explained by release of superoxide anion, endothelin-1, a COX product, or 20-HETE from the endothelium, or by electrotonic spread of excitability through myoendothelial gap junctions. As flow-mediated constriction in the rat carotid artery occurs at rates of flow found *in vivo*, the possibility that it may function as an extracranial mechanism to limit cerebral blood flow warrants further investigation.

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Conflicts of interest

No conflicts of interest are declared by the authors.

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