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PHARMACOLOGICAL IMPLICATIONS OF THE FLOW-DEPENDENCE OF VASCULAR SMOOTH MUSCLE TONE

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INTRODUCTION

The simplest model of the circulation consists of a pump—the heart—and branching arterial and venous systems that connect with each other through the microcirculation. These branching systems constrict and dilate in response to a variety of stimuli. The patterns and mechanisms of these changes are quite variable and at least some of them differ between vascular beds. Drugs can influence all components of the model, often in a complex way. This model is commonly used in pharmacological conceptualization; however, it does not include several regulating mechanisms inherent to the circulation that influence or modify its primary constrictor and dilator responses, including (*a*) the baroreceptor buffer mechanism, (*b*) myogenic (stretch) dependent changes in vascular tone, and (*c*) flow-initiated vasoconstriction and dilation.

The Baroreceptor Buffer Mechanism

This system senses changes in arterial pressure (and other vascular parameters) occurring in response to alterations in the external and internal

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environment and it coordinates through neural reflexes an overall, integrated, cardiovascular response (1).

Myogenic (Stretch) Dependent Vascular Tone Changes

This occurs predominantly in the medium and small arteries and arterioles—vessels that provide most of the active vascular resistance (2). An increase in intravascular pressure stretches the vascular smooth muscle cells and in response they contract; a decrease in pressure has an opposite effect. The efficiency of restoration of the vascular diameter is variable, depending on vessel size, location, and other considerations. This mechanism is also found in some veins. The responses to changes in intramural pressure occur irrespective of their cause and include those arising from drug administration. They can be viewed as a local vascular mechanism that protects the microvasculature from excessive increases or decreases in blood pressure.

Flow-initiated Vasoconstriction and Dilation

This mechanism is quite distinct from the myogenic stretch-initiated response (3). In vivo and in vitro studies of arteries of all sizes, and also of some veins, demonstrate that changes in intraluminal flow can alter vascular smooth muscle tone through a local mechanism (4). The most commonly observed response is dilation and both endothelial-dependent and -independent components have been recognized. Flow-related constriction has only been seen in vitro and can be elicited in both intact and endothelium-denuded arteries and veins. The flow response can be interpreted as an intrinsic local mechanism that allows intraluminal blood flow to modify and integrate the vascular tone of an entire branching system, in relation to the flow demand of the supplied tissue.

Although flow, presumably through shear stress, exerts a variety of effects on cells (5, 6), particularly those of the endothelium, this review deals exclusively with the short-term regulation of vascular tone, i.e. the influence of flow on the level of contraction of the vascular smooth muscle cell. A variety of long term effects of flow on the vascular wall have been proposed, including growth regulation and remodeling (7). Many of the current ideas on this topic and the changes that occur in disease have been summarized recently (8).

The short term consequences of the flow-sensitive vascular wall mechanisms on the overall cardiovascular responses to drugs are considered under two broad headings. The first deals with the modification of the primary pharmacological effect of a drug by the intrinsic flow-sensitive mechanisms in the blood vessel wall (Figure 1). Any drug that changes cardiac output or blood pressure is liable to cause changes in blood flow. This change in flow will itself alter vascular tone and as a result modify the primary direct

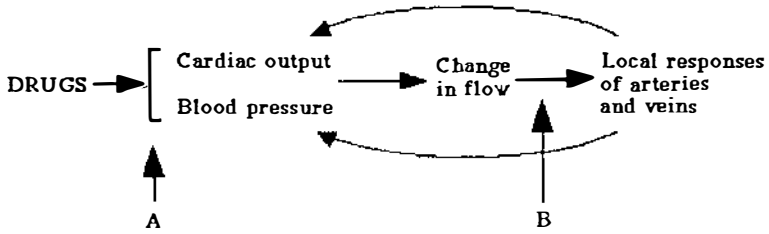

Figure 1 Two mechanisms of drug influence on flow-sensitive tone regulation: (*a*) modulation of the primary drug effect through a flow-sensitive mechanism and (*b*) site of pharmacological modification of the flow mechanism.

action of the drug. The second is the pharmacology of the flow mechanism itself. Flow involves many cellular processes that can be manipulated pharmacologically.

HISTORY OF THE FLOW-SENSITIVE EFFECT

Schretzenmayer (9) observed in 1933 that hyperemia of the dog hind limb dilates the femoral artery. This phenomenon has been repeatedly demonstrated in vivo in both large (10) and small arteries (11). Following the demonstration of the role of the endothelium in dilation to acetylcholine through the production of EDRF (12), flow-dilation in vivo has been shown to depend (at least in part) on the same mechanism (13). The endothelium component of flow-dilation depends on EDRF (14) and prostanoid (15) and is in part controlled by a sodium-sensitive flow sensor (4, 16–18). Both isometric (16–23) and isotonic (14, 24) experimental approaches have been adopted.

TECHNIQUES USED TO STUDY FLOW-DEPENDENT CHANGES IN VASCULAR TONE

Large artery flow diameter and intravascular pressure may be measured simultaneously in vivo in a vascular bed of an animal under anesthesia. Using an electromagnetic flow probe to measure blood flow in anesthetized dogs, Pohl et al (13) concluded that endothelial integrity was essential for the flow-dilation of the proximal femoral artery that developed after peripheral dilation or arteriovenous shunt. A Doppler flowmeter was used to demonstrate flow-dilation of the basilar artery in rats (25). Using a Doppler flowmeter catheter, researchers observed that, in humans, flow-induced dilation of coronary arteries was lost in atherosclerotic vessels (26). Using

the same technique, Levenson et al (27) observed that platelet intracellular calcium content was positively related to shear stress. Utilizing X-ray microangiography of rabbit ear arteries it was demonstrated that EDRF is essential for the development of collateral flow following arterial occlusion (28, 29). To assess the regulation of skeletal muscle vascular tone by flow, a dual slit video microscope was adopted to measure red blood cell velocity in exteriorized cat sartorius muscle (30). A similar technique has been used extensively to show the participation of prostanoids in the dilation-induced flow in the rat cremaster muscle (15).

The effect of flow on vascular tone has been assessed in vitro in several ways. In situ perfusion of mesenteric arteries has shown that flow-dilation opposes myogenic contraction in rabbit mesenteric circulation (31). Flow through a pipette onto the surface of human vascular endothelial cells increased intracellular Ca^{2+} measured with FURA 2 (32). Using a chamber designed to study only laminar flow (which was probably not the case in the above example), researchers found that flow increased intracellular Ca^{2+} in bovine cultured aortic endothelial cells (33). Although both laminar and turbulent flow raised intracellular Ca^{2+}, Na^+ uptake was increased only by turbulent flow over isolated smooth muscle cells (34). Laminar flow over isolated single endothelial cells attached to the lip of a pipette increased K^+ current measured by whole cell patch clamp (35).

Infusion of physiological saline solution into the lumen of small arteries (16–19, 21–23) and veins (19, 20) mounted under isometric conditions has been used extensively to study the cellular and subcellular mechanisms of flow. This system has revealed that the endothelium is not the only determinant of the dilator response to flow (22, 23) and has led to the discovery of flow-contraction (21). In perfused large vessels, increases in flow rate also induced constriction (36). Flow through a micropipette located inside one of the perfusion cannulae demonstrated the importance of the endothelium in flow-dilation under these experimental circumstances (14).

MECHANISM AND NATURE OF FLOW-RESPONSES

The flow of blood through a vessel induces shear stress related to the drag between the thin stationary layer of fluid associated with the vessel wall and the outer layers of the moving fluid. Shear stress (τ) is a function of the fluid viscosity (μ), flow rate (Q), radius (r), and wall shear rate (j):

$$\tau = \mu j = 4\mu Q/\pi r^3.$$

Shear stress occurs in the longitudinal axis of the vessel and can be contrasted with the tangential strain exerted by pressure at right angles to the long axis of the vessel. In vitro experiments can readily dissociate the

two effects. Shear stress may cause a conformational change in a surface macromolecule, resulting in alterations in transsarcolemmal ion flux. Changes associated with or resulting from these effects are responsible for the cellular response (19).

Alternatively, flow might modulate the level of intracellular calcium in vascular endothelial cells resulting from purinoreceptor activation. ATP at the surface of the endothelial cells is catabolized rapidly by nucleotidases and its local concentration may be flow-sensitive (37). This mechanism, however, depends on the continuous presence of ATP at the cell surface, which is unlikely to occur in vitro study.

Endothelium-Dependent Response to Flow

Flow-induced dilation in vessels of all sizes depends, at least in part, on the integrity of the vascular endothelium. Flow causes endothelial cells to elongate along the line of flow (38) and induces the release of EDRF from cultured cells (39), as well as from intact vessels in vivo (15, 40, 41) and in vitro vessel segments (14, 23, 24, 31). Flow induces an increase in intracellular free calcium (42), which is greater in the presence of ATP (43, 44) and it activates a K^+ current (35). It is presumably the increase in $Ca^{2+}{}_i$ that activates the NO-synthase and leads to EDRF release (45). In human endothelial cells, flow induces the production of diacylglycerol (46) and inositol-1,4,5-triphosphate (IP_3) (47), supporting the hypothesis that the sarcoplasmic reticulum takes part in the response to flow by releasing Ca^{2+} (42). In the rat cremaster muscle, flow induces the production of prostanoid (15).

Endothelium-independent Response to Flow

Removal of the endothelium or treatment with the NO-synthase inhibitors L-NNA or L-NAME decreases median levels of flow-induced dilation by about 30% in rabbit cerebral resistance (23, 48) and small ear arteries (22). In vivo, basilar artery blood flow was not influenced by topically applied L-NNA (25). In all the above experiments, indomethacin did not change flow-induced dilation. These results indicate that a significant component of this flow effect depends on mechanisms located in the subendothelium. Flow-induced contraction is completely independent of the endothelium in isometrically mounted resistance arteries (17, 21) and veins (20), as well as in perfused resistance (48, 49) and conduit arteries (36). Both responses to flow-constriction and dilation may be observed in the same isolated blood vessel. Under isometric conditions, in an artery with a high level of tone, flow induces dilation, whereas in one with a low level of tone, constriction is seen. An intermediate tone level can be found when no mean change in tone occurs with flow. This level is considered the null- or set-point when

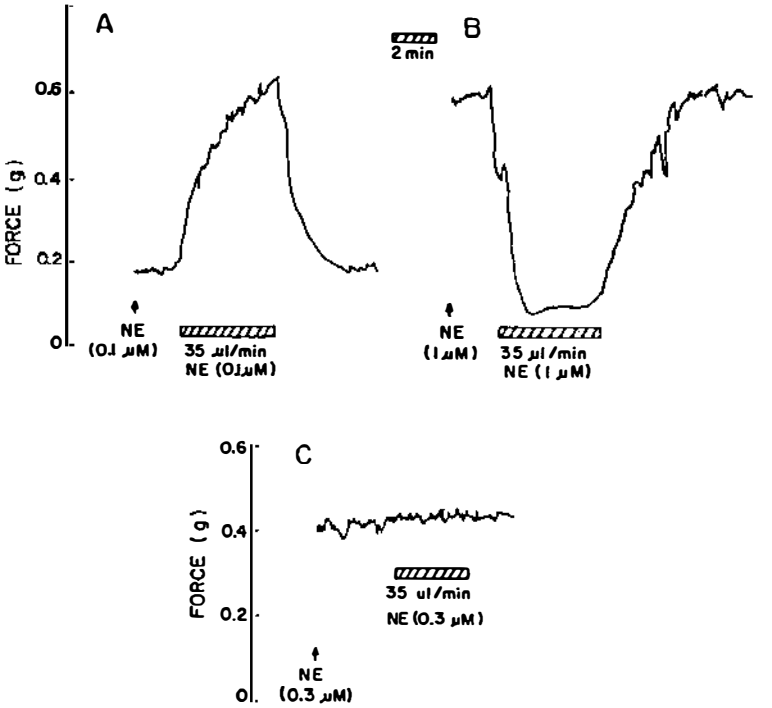

Figure 2 Response to intraluminal flow (hatched bars) of rabbit ear artery ring segments (outer diameter 150 μM) mounted isometrically in a myograph. Concentration of norepinephrine (NE) is shown below arrows. A: Flow into an artery with a low level of NE-induced tone. B: Flow into an artery with a high level of NE-induced tone. C: Flow into an artery with an intermediate level of NE-induced tone (from reference 50).

the opposing flow-responses are in balance (50) (Figure 2). Electrophysiological studies havc confirmed this balance effect (51). In the rabbit pial artery, the null-point, corresponding to an absence of flow response, occurs with a membrane potential of around –58 mV. Cells with a lower (i.e. less negative) membrane potential show hyperpolarization and those with higher (i.e. more negative) values exhibit depolarization with flow (51).

Flow-induced dilation and constriction are similarly attenuated by small decreases in extracellular Na^+ (16, 17, 52). Small decreases and increases in Na^+ decrease and increase, respectively, flow-contraction in the rabbit facial vein (D Henrion & JA Bevan, unpublished data). Such changes in [Na^+] have no effect on agonist-induced constrictor tone nor on acetylcholine- or papaverine-induced dilation (16, 17). Flow-induced contraction is associated with an increased $^{22}Na^+$ uptake (D Henrion et al, unpublished

data). Both flow-responses (16; D Henrion et al, unpublished data) share a similar sensitivity to the Na^+-H^+ exchange blocker amiloride. Interestingly, reducing Ca^{2+} up to 60% of its control value reduced the size of flow-dilation by the same amount as a similar percentage decrease in extracellular Na^+. When both Na^+ and Ca^{2+} were decreased together by the same percentage, flow-dilation remained unchanged, indicating that the Na^+/Ca^{2+} ratio is important in the flow mechanism (17). The parallel and equivalent effects of these experimental conditions on the two flow responses indicates that they reflect change at a common site, possibly the flow sensor (17).

The Physiological Advantage of Separate and Independent Vascular Responses to Changes in Intravascular Pressure and Flow

The tunica media of the entire arterial and venous systems contain sufficient muscle to influence local diameter at pressures considerably in excess of those considered physiological. This implies that vascular adjustments can potentially include changes in the diameter of blood vessels of all sizes. Vascular diameter is considered to reflect a compromise between the frictional resistance to flow—the smaller the diameter, the greater the resistance to flow—and the energy demands of a vascular system with a greater diameter that would be associated with a greater blood volume and a greater mass of vascular tissue (53). It has been hypothesized that when changes in blood flow to a vascular bed take place, to maximize efficiency, changes in diameter should take place in all the components of the branching system (54). Arteries of all sizes are able to alter their diameter in response to changes in flow (39, 40, 55). Responses to flow have also been reported in several veins (19, 20).

Although blood flow occurs only as a result of a pressure gradient, a vascular system that monitors and regulates itself only in response to changes in pressure cannot efficiently match cardiac output and blood pressure to varying tissue needs. The following example illustrates the physiological necessity of independent regulation of vascular pressure and flow. Peripheral flow can change as a result of an elevation of central arterial pressure (i.e. the perfusion pressure to a vascular bed) following increased cardiac output, or from peripheral resistance decrease caused, for example, by increased perivascular dilator nerve activity or increased tissue demand. In the first instance, there is a rise in central arterial pressure and in the pressure gradient between the central arteries and the smaller resistance arteries, potentially resulting in increased flow. In the second instance, although central pressure remains unchanged, resistance artery dilation leads to increased flow. In the first case, the flow increase would tend to occur in all vascular beds, it would be associated with an increase in pressure, and

it would be modified by local mechanisms. In the latter case, the increased demand could be met without an increase in central blood pressure and might be limited to one bed.

MODULATION OF THE PRIMARY VASCULAR RESPONSE BY LOCAL FLOW MECHANISM

There are currently no experimental studies of the extent to which local flow-sensitive vascular wall mechanisms modify the primary vascular change caused by physiological and pharmacological perturbations. However, we have included in this review two sets of data that support the existence of such mechanisms. The first set of data (see Table 1) provides a spectrum of some of the blood flow changes observed in animals and man under various experimental conditions. These are *end-flow* changes and presumably represent the primary effects of the initial maneuver, modified by vascular wall flow and stretch-sensitive mechanisms. Figure 2 summarizes quantitative data on blood vessel wall response to flow change. It seems clear from a comparison of Table 1 and Figure 2 that the flow-sensitive mechanism in the artery wall is sufficiently sensitive to respond significantly to changes in flow that occur with the administration of drugs and a variety of physiological circumstances.

The Dimension of Blood Flow-induced Changes with Some Physiological Stresses and Pharmacological Agents

Table 1 gives examples of changes in flow that have been recorded experimentally under various conditions in animals and humans (56–66). These measurements were made when presumably homeostatic mechanisms were operative. The primary changes are probably smaller than they would be in the absence of such regulatory mechanisms (see above). The greatest reported change is in the tongue of the dog, which showed a 1500% flow increase during heat stress (67). Skin flow showed an almost 600% increase due to calcitonin gene-related peptide (CGRP) (57) and an 80% decrease due to endothelin-1 (66). Because the cutaneous bed does not autoregulate effectively, these latter values may represent responses that are relatively unmodified by these buffering mechanisms. Other interesting examples include a 400% increase in hind limb flow due to exercise (64), a 300% increase in cerebral blood flow with hypercapnia (68), and in the human, an almost 500% increase in forearm blood flow with emotional stress (60). Endothelin-1 can reduce cutaneous flow to 20% of the control value (66).

Table 1 Changes in in vivo blood flow measured in response to various treatments in animals and humans

Species	Blood Flow (BF)	Agent	% Change in BF	Reference
Human	Coronary BF	Acetylcholine 0.5μM	+41%	56
Human	Carotid artery BF	CGRP	+52%	57
Human	Skin BF	CGRP	+582%	57
Human	Forearm BF	Norepineprine	−52%	58
Human	Forearm BF	Phenoxybenzamine	+190%	59
Human	Forearm BF	Emotional Stress	+490%	60
Goat	Coronary BF	L-NAME	−28%	61
Goat	Coronary BF	SNP	+325%	61
Goat	Cerebral BF	5-HT	−45%	62
Dog	Coronary BF	Glibenclamide	+55%	63
Dog	Hindlimb BF	Exercise	+408%	64
Dog	Cerebral BF	Enkephalin	+110%	65
Dog	Skin BF	Enkephalin	−67%	65
Rat	Skin BF	Endothelin-1	−80%	66
Rat	Skin BF	L-NAME	−46%	66
Dog	Tongue BF	Heat Stress	+1500%	67
Rat	Cerebral BF	Hypercapnia	+300%	68

Dimensions of the Flow-induced Change in the Blood Vessel Wall

There is a positive linear correlation between flow velocity and vessel diameter ($y = 0.176x + 4.589$, $r^2 = 0.540$, $n = 21$). This relation has been established from observations obtained both in vivo or in vitro in vessels with diameters of 30–185μm and with flow velocities of 3–45mm/sec (4, 24–26, 30, 31, 48, 49). Of particular interest to this review are the diameter changes that occur with flow change. A positive linear relationship exists between the log of the percent change in flow velocity and the percent change in vessel diameter under specific experimental conditions ($y = 17.92x - 24.65$, $r^2 = 0.507$, $n = 28$). Data are from in vivo measurement or from in vitro experiments where several flow rates have been tested (Figure 3). Several studies (14, 48, 69, 70) have shown that diameter reaches a new plateau within several minutes of flow change.

PHARMACOLOGY OF THE LOCAL FLOW MECHANISM

Vascular Endothelial Cells

The vascular endothelium is the first target cell reached by circulating pharmacological agents. Endothelial cell production of NO is attenuated by

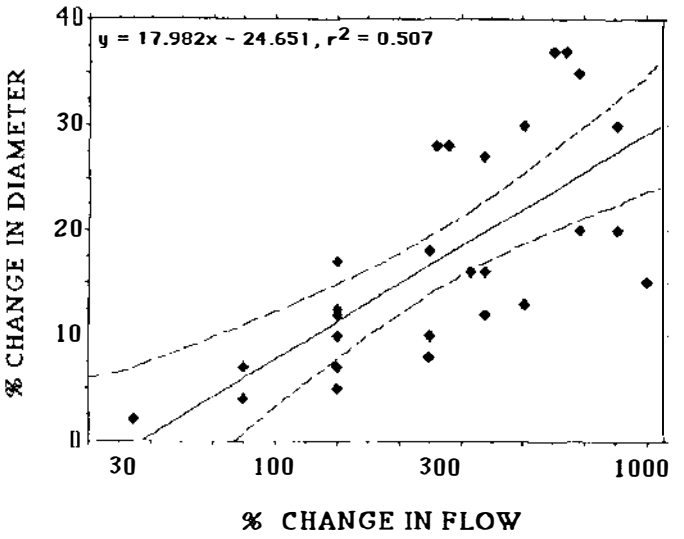

Figure 3 Relationship between change of flow and the consequent changes in diameter of blood vessels from different vascular beds, species and under various experimental circumstances. For details see 15, 24–26, 30, 31, 36, 40, 48, 49, 70, 71, 87–92.

NO-synthase inhibition. L-NNA perfusion in conscious dogs raises blood pressure and decreases heart rate (71), probably by modifying a continuous NO production induced at least in part by flow itself. L-NAME suppresses flow-dilation in isolated cannulated microvessels of the porcine heart (80–140 μm) (24), but L-NNA and L-NAME only attenuate flow-dilation in the rabbit pial artery (23). These agents had no effect on flow-dilation after endothelium removal. In addition, flow-mediated release of EDRF is suppressed by methylene blue, which inhibits cGMP synthesis; oxyhemoglobin, which is a NO scavenger; and by the removal of Ca^{2+}, which is required for NO production (72). The calcium entry pathway for endothelium-dependent flow-dilation has a different pharmacological spectrum from the voltage-dependent calcium channel (XW Xiao & JA Bevan, submitted). Olesen et al (35) have shown a shear stress–induced K^+ current in endothelial cells. Several blockers of K^+ channels have been tested in relation to flow, including glibenclamide, blockers of ATP-sensitive K^+ channels (TEA; 10–5M), and charybdotoxin, a blocker of Ca^{2+}-activated K^+ channels. $^{86}Rb^+$ efflux resulting from shear stress in calf pulmonary artery endothelial cells is not affected by TEA or charybdotoxin (73). In the rat basilar artery, in vivo topical application of neither TEA nor glibenclamide blocked flow-dilation (25). Pharmacological evidence suggests that the endothelial component of flow-dilation in the rabbit pial artery is associated with the activation

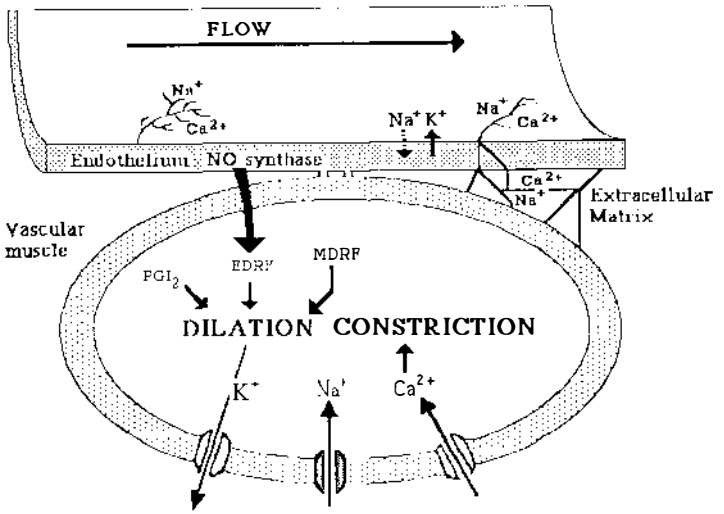

Figure 4 Schematic representation of some of the mechanisms underlying the changes that occur in smooth muscle tone of a blood vessel with alteration in intraluminal flow. Other mechanisms have been described in specific blood vessels. (See text for details.)

of the internal rectifying K channel. The endothelium-mediated response to shear stress (73) probably involves a different pathway from the non-endothelium–dependent response (25, 73; GC Wellman & JA Bevan, submitted) (see figure 4).

Endothelial cells are also able to produce and release cyclooxygenase products such as PGI_2 with flow stimulation. In the rat cremaster muscle, indomethacin attenuates the functionally important flow-dilation (15, 74).

Endothelin-1-induced contraction is increased in the isolated femoral artery upon flow, resulting from either a decrease in its stores or an increased receptor sensitivity, or both (75). One pharmacological implication of a flow-induced decrease in endothelin-1 production would be an attenuation of the PKC-dependent potentiation by ET-1 of the vascular contractile response to agonists. Indeed, vascular reactivity to norepinephrine is greater upon activation of PKC by sub-threshold concentrations of ET-1 compatible with measured plasma levels (76).

Vascular Smooth Muscle Cells

Flow dilation in some blood vessels has both an endothelium-dependent (see above) and an endothelium-independent component (3, 22). The latter does not involve NO production, cycloxygenase products, or neurogenic

mediators (23, 25). Likewise, flow-induced contraction is totally independent of the endothelium, both in arteries (3, 36, 49, 54) and in veins (20). (See figure 4.)

Drugs that Influence Ionic Mechanisms

Substances affecting Na^+-dependent mechanisms also inhibit flow-dilation. Blockers of Na^+-H^+ exchange, such as amiloride or methyl-isobutyl-amiloride, attenuate both flow-dilation (16) and contraction. Monensin, a Na^+ ionophore, inhibits both responses (16). The similarity of pharmacological effects on the opposite vascular responses to flow is consistent with the hypothesis that the sodium-related similarity reflects the sensitivity of a common pathway (4, 18, 77). (See figure 4.)

Flow-induced contraction is associated with the entry of Ca^{2+}: It is suppressed in ring segments of the facial vein bathed in a medium without Ca^{2+} or EGTA (20). Ca^{2+} entry blockers such as nifedipine, diltiazem, and verapamil attenuate flow-contraction in a dosage similar to potassium-induced contraction, indicating that voltage-dependent Ca^{2+} entry is important in this response (78). Myogenic and agonist-induced contraction have different pharmacological profiles (78). Supporting this conclusion are findings that flow-contraction is potentiated by the Ca^{2+} channel activator Bay K 8644 (D Henrion & JA Bevan, unpublished observation), and is associated with vascular smooth muscle cell depolarization (51).

GENERAL PHARMACOLOGICAL IMPLICATIONS OF THE FLOW-REGULATION OF VASCULAR TONE

The Importance of Vascular Tone Level

Isometrically mounted vessel segments without myogenic tone have been used to analyze the mechanism of the flow effect on vascular smooth muscle tone. Flow initiates two concurrent but opposite responses (50), reflecting independent cellular mechanisms. Supporting this theory are observations that chronic sympathetic denervation (79) augments flow contraction and diminishes flow-dilation.

In the first series of experiments indicating independent mechanisms (50), when the level of wall tone was changed by exposure to different concentrations of norepinephrine (0–90% of tissue maximum), flow tended to shift wall tone toward an intermediate point. When the wall tone was set at this intermediate level by adjusting the norepinephrine concentration, flow had little effect on wall tone, although oscillations, presumably reflecting this interaction, often were observed. This balance point varied between different vascular segments. An electrophysiological study (51) supports this concept.

Thus, changes in the blood vessel tone level would be expected to qualitatively and quantitatively influence the response to flow. Also, if the administered drug causes concomitant changes in arterial pressure by altering the tone level, and these changes are associated with a change in membrane potential, then they would be expected to modify the response to flow.

In vivo studies of the circulation are usually undertaken without considering such possible complications. This does not matter when only the end result of the pharmacological manipulation is measured; however, such issues should be considered in detailed, functional mechanistic studies.

Theoretical Implications of the Vascular Wall Flow Sensitive Mechanism

UPSTREAM DRUG ACTION WITH POSSIBLE DOWNSTREAM CONSEQUENCES In the brain circulation, at least in rabbits and humans, α-adrenoceptors are restricted to the origin and main branches of the large cerebral arteries (81). Smaller vessels respond minimally or not at all to α-adrenergic agents or antagonists. Upstream constriction could cause downstream changes in caliber, resulting in changes in blood flow. This theory is consistent with much of the literature on the sympathetic control of cerebral blood flow (81, 82).

UPSTREAM CONSEQUENCES ON VASCULAR TONE OF DOWNSTREAM DRUG ACTION The intraarterial injection of a vasodilator drug into a distal artery or the initiation of metabolic dilation downstream can cause flow changes in the upstream supply artery. An experimental approach based on this concept resulted in the first documented demonstration of flow-initiated vascular regulation (9). This approach has been used subsequently in both animals and man (15, 26, 55). Thus, an observed change in vascular diameter after drug administration in vivo does not necessarily implicate local drug action.

ACETYLCHOLINE AND FLOW-DILATION Many researchers tacitly assume that endothelium-dependent acetylcholine dilation reflects the shear stress–induced responses of the vasculature, but this is not necessarily the case. Acetylcholine causes the release of EDRF(s) from the endothelium (12), but the response to flow is much more complex than this (see above). The flow sensor and the muscarinic endothelial receptor are unlikely to coexist throughout the circulation. That they reflect each other becomes even less likely in the circulation damaged by disease.

IN VITRO AND IN VIVO DRUG DIFFERENCES There are many reasons why the action of a drug in vitro may differ from that in vivo. On the one hand,

the presence of a flow-sensitive system provides an additional basis for discrepancy, especially if the in vitro preparation involves a static, non-flow state. On the other hand, responses of an in vitro tissue system that involves perfusion might include flow effects. Further discrepancy might result from the difference between the viscosity of perfusion fluids and blood.

DRUG EFFECT IS DEPENDENT ON THE ARTERIAL PRESSURE There are reports that drug effects in vivo differ quantitatively and qualitatively with the level of the arterial pressure (83–86). This is not surprising in view of the buffer pressoreceptor nerves, and the presence of stretch-dependent tone in resistance arteries. The fact that the vascular flow–sensitive mechanism leads to a response that is also dcpcndcnt on vascular tonc lcvcl adds further complexity.

SUMMARY

The recognition that the wall tone of most arteries and veins can change in response to shear stress has several implications for our understanding of the effects of drugs on the circulation. By a primary action on the heart and vasculature, drugs can cause changes in cardiac output and blood pressure that lead to changes in blood flow. These changes in blood flow can secondarily change vascular diameter, thus complicating the basic response. Furthermore, drugs can modify the local flow-sensitive mechanism directly. The flow-initiated effect seems to depend, both qualitatively and quantitatively, on the level of wall tone and is not entirely endothelium-dependent. If the primary action of a drug is to alter the tone level of vascular smooth muscle directly or if tone changes as a result of a change in blood pressure (and thus in local myogenic control), then it follows that these changes in turn influence the flow response, both quantitatively and qualitatively. The vascular response to flow is complex both in its site of origin and the functional changes initiated. It is not synonymous with the endothelial-dependent action of acetylcholine.

Literature Cited

1. Heymans C, Neil E. 1958. *Areas of the Cardiovascular System*. London: Churchill
2. Johnson PC. 1986. Autoregulation of blood flow. *Circ. Res.* 59:483–95
3. Bevan JA, Laher I. 1991. Pressure and flow-dependent vascular tone. *FASEB J.* 5:2267–73
4. Bevan JA. 1991. Flow-dependent vascular tone. In *The Resistance Vasculature*, ed. JA Bevan, W Halpern, MJ

Mulvany, pp. 169–91. Clifton, NJ: Humana
5. Davies PF. 1989. How do vascular endothelial cells respond to flow? *News Physiol. Sci.* 4:22–25
6. Nerem RM, Girard PR. 1990. Hemodynamic influences on vascular endothelial biology. *Toxicol. Pathol.* 18: 572–82
7. Langille BL. 1993. Flow-dependent remodelling of developing and mature arteries. See Ref. 8. In press
8. Bevan JA, Kaley G, Rubanyi GM, eds. 1993. *Flow Dependent Regulation of Vascular Function*. Oxford: Oxford Univ. Press. In press
9. Schretzenmayer A. 1933. Uber kreislaufregulatorische vorgange an der grossen arterien bei muskularbeit. *Pflüger Arch. Gesamte Physiol. Menschen Tiere* 232:743–48
10. Lie M, Sejersted DM, Kiil F. 1970. Local regulation of vascular cross section during changes in femoral arterial blood flow in dogs. *Circ. Res.* 27:727–37
11. Johnson PC, Intaglietta M. 1976. Contributions of pressure and flow sensitivity to autoregulation in mesenteric arterioles. *Am. J. Physiol.* 231:1686–98
12. Furchgott RF, Zawadzki JV. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373–76
13. Pohl V, Holtz J, Busse R, Bassenge E. 1986. Crucial role of endothelium in the vasodilator response to increased flow *in vivo*. *Hypertension* 8:37–44
14. Kuo L, Davis MJ, Chillian DW. 1990. Endothelium-dependent, flow-induced dilation of isolated coronary arterioles. *Am. J. Physiol.* 259:H1063–70
15. Koller A, Kaley G. 1990. Prostaglandins mediate arteriolar dilation to increased blood flow velocity in skeletal muscle microcirculation. *Circ. Res.* 67:529–34
16. Bevan JA, Joyce EH. 1990. Flow induced relaxation in a resistance artery is associated with an amiloride sensitive sodium-dependent mechanism in vascular smooth muscle. *J. Vasc. Med. Biol.* 2:281–88
17. Bevan JA, Joyce EH. 1992. Comparable sensitivity of flow contraction and relaxation to Na reduction may reflect flow-sensor characteristics. *Am. J. Physiol.* 263:H182–187
18. Bevan JA, Siegel G. 1991. Blood vessel wall matrix flow sensor: support and speculation. *Blood Vessels* 28:552–56
19. Bevan JA, Joyce EH. 1990. Saline infusion into lumen of resistance artery and small vein causes contraction. *Am. J. Physiol.* 259:H23–28
20. Henrion D, Laher I, Bevan JA. 1992. Intraluminal flow increases vascular tone and $^{45}Ca^{2+}$ influx in the rabbit facial vein. *Circ. Res.* 71:339–45
21. Bevan JA, Joyce EH. 1988. Flow-dependent contraction observed in a myograph-mounted resistance artery. *Blood Vessels* 25:261–64
22. Bevan JA, Joyce EH, Wellman GC. 1988. Flow-dependent dilation in a resistance artery still occurs after endothelium removal. *Circ. Res.* 63:980–85
23. Gaw AJ, Bevan JA. 1993. Flow-induced relaxation of the rabbit middle cerebral artery is composed of both endothelium-dependent and -independent components. *Stroke* 24:105–10
24. Kuo LIH, Chilian WH, Davis MJ. 1991. Interaction of pressure- and flow-induced responses in procine coronary resistance vessels. *Am. J. Physiol.* 260:H1706–15
25. Fujii K, Heistad DD, Faraci FM. 1991. Flow-mediated dilatation of the basilar artery in vivo. *Circ. Res.* 69:697–705
26. Nabel EG, Selwyn AP, Ganz P. 1990. Large coronary arteries in humans are responsive to changing blood flow. An endothelium-dependent mechanism that fails in patients with atherosclerosis. *J. Am. Coll. Cardiol.* 16:349–56
27. Levenson J, Devynck M-A, Pithois-Merli I, Sang KHLQ, Filitti V, Simon A. 1990. Dynamic association between artery shear flow condition and platelet cytosolic free Ca^{2+} concentration in human hypertension. *Clin. Sci.* 79:613–18
28. Randall MD, Griffith TM. 1992. Collateral perfusion: the role of endothelium-derived relaxing factor and effects of vasodilators. *J. Cardiovasc. Pharmacol.* 20:S166–69
29. Randall MD, Griffith TM. 1992. EDRF plays central role in collateral flow after arterial occlusion in rabbit ear. *Am. J. Physiol.* 263:H752–60
30. Ping P, Johnson PC. 1992. Role of myogenic response in enhancing autoregulation of flow during sympathetic nerve stimulation. *Am. J. Physiol.* 263: H1177–84
31. Pohl Y, Herlan K, Huang A, Bassenge E. 1991. EDRF-mediated shear-induced dilation opposes myogenic vasoconstriction in small rabbit arteries. *Am. J. Physiol.* 261:H2016–23
32. Schwartz G, Froogmans G, Nilius B.

1992. Shear stress induced membrane currents and calcium transients in human vascular endothelial cells. *Pflügers Arch.* 421:394–96
33. Ando J, Komatsuda T, Kamiya A. 1988. Cytoplasmic calcium response to fluid shear stress in cultured vascular endothelial cells. *In Vitro Cell Dev. Biol.* 24:871–77
34. Rosati C, Garay R. 1991. Flow-dependent stimulation of sodium and cholesterol uptake and cell growth in cultured vascular smooth muscle. *J. Hypertens.* 9:1029–33
35. Olesen SP, Clapham DE, Davies PF. 1988. Haemodynamic shear stress activates a K^+ current in vascular endothelial cells. *Nature* 331:168–70
36. Sipkema P, van der Linden PJW, Hoogerwerf N, Westerhof N. 1989. Does the endothelium play a role in flow-dependent contraction? *Blood Vessels* 26:368–76
37. Dull RO, Tarbell JM, Davies PF. 1992. Mechanisms of flow-mediated signal transduction in endothelial cells: kinetics of ATP surface concentrations. *J. Vasc. Res.* 29:410–19
38. Langille BL, Adamson SL. 1981. Relationship between blood flow direction and endothelial cell orientation at arterial branch sites in rabbits and mices. *Circ. Res.* 48:481–88
39. Cooke JP, Stamler J, Andon N, Davies PF, McKinley G, Loscalzo J. 1990. Flow stimulates endothelial cells to release a nitrovasodilator that is potentiated by reduced thiol. *Am. J. Physiol.* 259:H804–12
40. Koller A, Messina EJ, Wolin MS, Kaley G. 1989. Effects of endothelial impairment on arteriolar dilator responses in vivo. *Am. J. Physiol.* 257: H1485–89
41. Koller A, Kaley G. 1990. Role of endothelium in reactive dilation of skeletal muscle arterioles. *Am. J. Physiol.* 259:H1313–16
42. Shen J, Luscinskas FW, Connolly A, Dewey CF Jr, Gimbrone MA Jr. 1992. Fluid shear stress modulates cytosolic free calcium in vascular endothelial cells. *Am. J. Physiol.* 262:C384–90
43. Mo M, Eskin SG, Schilling WP. 1991. Flow-induced changes in Ca^{2+} signaling of vascular endothelial cells: effect of shear stress and ATP. *Am. J. Physiol.* 260:H1698–1707
44. Dull RO, Davies PF. 1991. Flow modulation of agonist (ATP)-response (Ca^{2+}) coupling in vascular endothelial cells. *Am. J. Physiol.* 261:H149–54
45. Moncada S, Palmer RMJ, Higgs EA. 1991. Nitric oxide: physiology, physiopathology and pharmacology. *Pharmacol. Rev.* 43:100–42
46. Hsieh HJ, Li NQ, Frangos JA. 1992. Shear-induced platelet-derived growth factor gene expression in human endothelial cells is mediated by protein kinase C. *J. Cell. Physiol.* 150:552–58
47. Nollert MV, Diamond SL, McIntire LV. 1991. Hydrodynamic shear stress and mass transport of endothelial cell metabolism. *Biotechnol. Bioeng.* 38: 588–602
48. Garcia-Roldan JL, Bevan JA. 1991. Augmentation of endothelium-independent flow constriction in pial arteries at high intravascular pressures. *Hypertension* 17:870–74
49. Hoogerwerf N, van der Linden PJW, Westerhof N, Sipkema P. 1992. A new mounting technique for perfusion of isolated small arteries: the effects of flow and oxygen on diameter. *Microvasc. Res.* 44:49–60
50. Bevan JA, Joyce EH. 1990. Flow-induced resistance artery tone: balance between constrictor and dilator mechanisms. *Am. J. Physiol.* 258:H663–68
51. Bevan JA, Wellman GC. 1993. Intraluminal flow-initiated hyperpolarization and depolarization shift the membrane potential of arterial smooth muscle towards an intermediate level. In press
52. Henrion D, Klaasen A, Bevan JA. 1992. Extracellular sodium modulates flow and stretch-induced contraction in opposite directions (abstract). *FASEB J.* 6:A1545
53. Zamir M. 1977. Shear forces and blood vessel radii in the cardiovascular system. *J. Gen. Physiol.* 69:449–61
54. Sherman TF. 1981. On connecting large vessels to small. *J. Gen. Physiol.* 78:431–53
55. Hintze TH, Vatner SF. 1984. Reactive dilation of large coronary arteries in conscious dogs. *Circ. Res.* 54:50–57
56. Dubois-Rande JL, Zelinsky R, Roudot F, Chabrier PE, Castaigne A, et al. 1992. Effects of infusion of L-arginine into the left anterior descending coronary artery on acetylcholine-induced vasoconstriction of human atheromatous coronary arteries. *Am. J. Cardiol.* 70:1269–75
57. Jager K, Muench R, Seifert H, Beglinger C, Bollinger A, Fischer JA. 1990. Calcitonin gene-related peptide (CGRP) causes redistribution of blood flow in humans. *Eur. J. Clin. Pharmacol.* 39:491–94
58. Cockcroft JR, Clarke JG, Webb JDJ.

1991. The effect of intra-arterial endothelin on resting blood flow and sympathetically mediated vasoconstriction in the forearm of man. *Br. J. Clin. Pharmacol.* 31:521–24

59. Kahan T, Taddei S, Pedrinelli R, Hjemdahl P, Salvetti A. 1992. Non-adrenergic sympathetic vascular control of the human forearm in hypertension: possible involvement of neuropeptide Y. *J. Cardiovasc. Pharmacol.* 19:587–92
60. Blair DA, Glover WE, Greenfield ADM, Roddie LC. 1959. Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress. *J. Physiol.* 148:633–47
61. Garcia JL, Fernandez N, Garcia-Villalon AL, Monge L, Gomez B, Dieguez G. 1992. Effects of nitric oxide synthesis inhibition on the goat coronary circulation under basal conditions and after vasodilator stimulation. *Br. J. Pharmacol.* 106:563–67
62. Gomez B, Garcia-Villallon AL, Frank A, Garcia JL, Monge L, Dieguez G. 1992. Effects of hypoglycemia on the cerebral circulation in awake goats. *Neurology* 42:909–16
63. Imamura Y, Tomoike H, Narishige T, Takahashi T, Kasuya H, Takeshita A. 1992. Glibenclamide decreases basal coronary blood flow in anesthetized dogs. *Am. J. Physiol.* 263:H399–404
64. Donald DE, Rowlands DJ, Ferguson DA. 1970. Similarity of blood flow in the normal and the sympathectomized dog hindlimb during grades exercise. *Circ. Res.* 26:185–99
65. Rosen CL, Cote A, Haddad GG. 1989. Effect of enkephalins on cardiac output and regional blood flow in conscous dogs. *Am. J. Physiol.* 256:H1651–58
66. Lawrence E, Brain SD. 1992. Responses to endothelin in the rat cutaneous microvasculature: a modulatory role of locally-produced nitric oxide. *Br. J. Pharmacol.* 106:733–38
67. Hales JRS, Dampney RAL. 1975. The redistribution of cardiac output in the dog during heat stress. *J. Therm. Biol.* 1:29–34
68. Dora E, Hines K, Kunos G, McLaughlin AC. 1992. Significance of an opiate mechanism in the adjustment of cerebrocortical oxygen consumption and blood flow during hypercapnic stress. *Brain Res.* 573:293–98
69. Munge A, Heublein B, Kuhn M, Nolte C, Haverich A, et al. 1993. Impaired coronary dilator responses to substance P and impaired flow-dependent dilator responses in heart transplant patients with graft vasculopathy. *J. Am. Coll. Cardiol.* 21:163–70
70. Wang J, Zeballos GA, Kaley G, Hintze TH. 1991. Dilation and constriction of large coronary arteries in conscious dogs by endothelin. *Am. J. Physiol.* 261:H1379–86
71. Persson PB, Baumann JE, Ehmke H, Nafz B, Wittmann U, Kirchheim HR. 1992. Phasic and 24-h blood pressure control by endothelium-derived relaxing factor in conscious dogs. *Am. J. Physiol.* 262:H1395–1400
72. Buga GM, Gold ME, Fukuto JM, Ignarro LJ. 1991. Shear stress-induced release of nitric oxide from endothelial cells grown on beads. *Hypertension* 17:187–93
73. Schilling WP, Mo M, Eskin SG. 1992. Effect of shear stress on cytosolic Ca^{2+} of calf pulmonary artery endothelial cells. *Exp. Cell. Res.* 198:31–35
74. Koller A, Kaley G. 1991. Endothelial regulation of wall shear stress and blood flow in skeletal muscle microcirculation. *Am. J. Physiol.* 260:H862–68
75. Miller VM, Burnett JC Jr. 1992. Modulation of NO and endothelin by chronic increases in blood flow in canine femoral arteries. *Am. J. Physiol.* 263:H103–8
76. Henrion D, Laher I. 1993. Potentiation of norepinephrine-induced contractions by endothelin-1 in the rabbit aorta. *Hypertension* 22:13–20
77. Bevan JA. 1993. Flow-induced contraction and relaxation: their role in the regulation of vascular tone results of in vitro studies. See Ref. 8. In press
78. Xiao XW, Bevan JA. 1993. Pharmacological evidence that flow- and potassium-induced contraction of rabbit facial vein may involve the same calcium entry pathway. *J. Pharmacol. Exp. Ther.* In press
79. Bevan RD, Clementson A, Joyce EH, Bevan JA. 1993. Sympathetic denervation of resistance arteries increases contraction and decreases relaxation to flow. *Am. J. Physiol.* H490–94
80. VanRiper DA, Bevan JA. 1991. Selective variation of agonist and neurally mediated vasoconstriction with rabbit middle cerebral artery branch order. *J. Pharmacol. Exp. Ther.* 257: 879–86
81. Bevan RD, Dodge JT, Wellman T, Walters CL, Bevan JA. 1993. Is there a neurogenic influence on the diameter of human small pial arteries? In *The Human Brain Circulation: Functional*

Changes in Disease, ed. JA Bevan, RD Bevan. Clifton, NJ: Humana
82. Bevan JA, Bevan RD. 1993. Is innervation a prime regulator of cerebral blood flow? *News Physiol. Sci.* 8:149–53
83. Harder DR, Gilbert R, Lombard JH. 1987. Vascular muscle cell depolarization and activation in renal arteries on elevation of transmural pressure. *Am. J. Physiol.* 253:F778–81
84. Henrion D, Egleme C, Criscione L, Wood JM. 1989. Blood pressure, the renin-angiotensin system and neurogenic vasoconstriction in pithed rats. *J. Pharm. Pharmacol.* 41:766–69
85. Harder DR. 1988. Increased sensitivity of cat cerebral arteries to serotonin upon elevation of transmural pressure. *Pflügers Arch. Eur. J. Physiol.* 411: 698–700
86. Lombard JH, Eskinder H, Kauser K, Osborn JL, Harder DR. 1990. Enhanced norepinephrine sensitivity in renal arteries at elevated transmural pressure. *Am. J. Physiol.* 259: H29–33
87. Vita JA, Treasure CB, Ganz P, Cox DA, Fish RD, Selwyn AP. 1989. Control of shear stress in the epicardial coronary arteries of humans: impairment by atherosclerosis. *J. Am. Coll. Cardiol.* 14:1193–99
88. Zelis R, Hayoz D, Drexler H, Munzel T, Hornig B, et al. 1992. Arterial dilatory reserve in congestive heart failure. *J. Hypertens.* 10:S65–67
89. Melkumyants AM, Balashov SA, Klimachev AN, Kartamyshev SP, Khayutin VM. 1992. Nitric oxide does not mediate flow induced endothelium dependent arterial dilatation in the cat. *Cardiovasc. Res.* 26:256–60
90. McLenachan JM, Williams JK, Fish RD, Ganz P, Selwyn AP. 1991. Loss of flow-mediated endothelium-dependent dilation occurs early in the development of atherosclerosis. *Circulation* 84:1273–78
91. Mellinger BC, Vaughan ED Jr. 1990. Penile blood flow changes in the flaccid and erect state in potent young men measured by duplex scanning. *J. Urol.* 144:894–96
92. Tohda K, Masuda H, Kawamura K, Shozawa T. 1992. Difference in dilatation between endothelium-preserved and -desquamated segments in the flow-loaded rat common carotid artery. *Arterioscler. Thromb.* 12:519–28