Invited Review

Spreading vasodilatation in resistance arteries

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Abstract

Focal application of vasodilators such as acetylcholine (ACh), which evoke arterial hyperpolarization, cause coordinated dilatation along the length of an artery with minimal decay with distance from the site of application. This phenomenon is called spreading vasodilatation. In an artery wall, the endothelium is separated from the surrounding smooth muscle cell layers by an internal elastic lamina (IEL). Adjacent endothelial cells are strongly connected via gap junctions, which can allow direct communication between the cells, including the passage of small molecules and electrical current. Direct communication between an endothelial cell and a smooth muscle cell, through a hole in the IEL, has recently been observed in arteries. Spreading vasodilatation is associated with a spread of hyperpolarization which may be a key mechanism responsible for this spreading arterial vasodilatation. Endothelial cells appear to play an important role in such spread, even though the facilitating mechanisms underlying this spread are as yet unclear. These spreading responses are likely to have an important physiological role in the coordination of blood flow within a vascular network.

Key words: spreading response, artery, vasodilatation, conduction

Introduction

August Krogh (1920) first reported that the local application of vasodilators could stimulate rapid and extensive vasodilatation at distant sites, which could not be explained simply on the basis of diffusion of the dilator agent. Conducted responses of this type are referred to as spreading vasodilatation, and have been mainly studied in the smallest arteries within the microcirculation. However, in the vascular bed of skeletal muscle, dilatation has been shown to ascend from the microcirculation to dilate feed arteries, increasing blood flow to meet the increased metabolic demand associated with muscle activity (Gustafsson *et al.*, 1999; Segal, 2005). Spreading responses are not restricted to vasodilatation, and have also been reported in some situations to be evoked with constrictor agents, although it is of interest that this type of spread does not appear to be as extensive as the equivalent vasodilatation. Although spreading responses have mainly been studied in the arterioles of the microcirculation, and skeletal

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muscle vasculature, recent reports show similar responses occur in larger resistance size arteries. This indicates that spread of vasodilatation is general characteristic of the vascular system and suggesting a key physiological role for the phenomenon.

Structure of arteries

Endothelium comprises a monolayer of endothelial cells in contact with the blood. These cells are elliptical in shape and are aligned along the longitudinal axis of an artery (Emerson *et al.*, 2000). Smooth muscle cells are more elongated, and effectively surround the endothelial monolayer with a variable number of layers depending on location and size of the vessel (Emerson *et al.*, 2000). For example, there are 3–4 layers of smooth muscle in the rat mesenteric artery branches we have used to study spreading vasodilatation (Sandow *et al.*, 2000), and in mouse mesenteric artery branches, there are about 2 layers (Dora *et al.*, 2003a). The internal elastic lamina lies between the smooth muscle and endothelial cell layers (Sandow *et al.*, 2002; Dora *et al.*, 2003a).

Cells comprising the artery wall are linked by physical contacts, gap junctions, which enable cell-cell communication. Gap junctions have characteristic pentalaminar structure at the border between two cells, and form large and extensive homocellular contacts between adjacent endothelial cells, while equivalent homocellular gap junctions between smooth muscle cells appear to be less extensive (Sandow *et al.*, 2000; Gustafsson *et al.*, 2003). Heterocellular gap junctions directly linking endothelial and smooth muscle cells are also present in some, but not all, arteries, and apparently in varying numbers correlating inversely with artery size. Characteristically, these 'myoendothelial' gap junctions (MEGJs) comprise an endothelial cell projection passing through a hole or perforation in the IEL to make direct contact with the membrane of a smooth muscle cell with a resultant pentalaminar appearance (Dora *et al.*, 2003a; Sandow *et al.*, 2000). Gap junctions are formed by two hemichannels called connexons, each comprising six transmembrane proteins or connexins (Cx). Several types of Cx have been identified in vascular tissue, with types Cx43 and Cx45 having been widely observed in vascular smooth muscle cells, (Rummery *et al.*, 2002; Li *et al.*, 2001; Ko *et al.*, 2001), and types Cx37, Cx40 and Cx43 in endothelial cells (Rummery *et al.*, 2002; Gustafsson *et al.*, 2003). Experimental injection of dye into a single cell shows that the gap junctions enable the cells in the artery wall to communicate directly, as dye spreads rapidly between endothelial cells, and between endothelial cells and the adjacent smooth muscle, but much less extensively between the smooth muscle cells (Little *et al.*, 1995; Emerson *et al.*, 2000; Dora *et al.*, 2003a; Takano *et al.*, 2004). There is also variation in the ability of different dyes to spread between cells. In hamster cheek pouch arterioles, lucifer yellow, a commonly used dye to mark impaled cells, did not spread between endothelial and smooth muscle cells, while biocytin, another neutral dye, did (Little *et al.*, 1995). However, the dye transferred more readily from endothelial cells to smooth muscle cells than in the opposite direction (Little *et al.*, 1995).

Ca^{2+} ion and inositol trisphosphate (IP_3) can also pass through gap junctions, and in arterioles these substances have been postulated to pass from activated smooth muscle to the endothelium and increase the release of the dilator nitric oxide (NO) (Dora, 2001a; Figueroa *et*

al., 2004). Gap junctions also provide a potential pathway for current spread between adjacent cells, which may be sufficient to alter the cell membrane potential in coupled cells. In guinea-pig arterioles, which are comprised of an endothelium and a single layer of smooth muscle cells, endothelial cell hyperpolarization evoked by either ACh or current injection was conducted to smooth muscle cells, and in addition, action potentials evoked in smooth muscle cells by Ba^{2+} spread to the endothelium (Yamamoto *et al.*, 2001). The conduction of endothelial cell hyperpolarization evoked with ACh was blocked with gap junction uncouplers (Yamamoto *et al.*, 1999).

Vasodilatation

A variety of chemicals can dilate blood vessels by relaxing the smooth muscle either directly or indirectly via the endothelium, and by different mechanisms. ACh is an agonist for muscarinic cholinergic receptors which are present on endothelial cells, but not usually on vascular smooth muscle. Stimulation of these receptors with ACh leads to endothelium-dependent vascular dilatation through one or a combination of three distinct pathways; 1) the release of endothelium-derived relaxing factor (EDRF), now known to be NO synthesized and released from the endothelium, which diffuses to and relaxes nearby smooth muscle cells (Rapoport *et al.*, 1983; Furchgott *et al.*, 1980); 2) prostaglandin I_2 (PGI_2) produced initially by cyclooxygenase activation (Gryglewski *et al.*, 1986; Zhao *et al.*, 1996); and 3) endothelium-derived hyperpolarization factor (EDHF), which underlies the persisting dilatation when NO synthase and cyclooxygenase inhibitors are present, and which is associated with smooth muscle cell hyperpolarization (Chen *et al.*, 1988). The basic nature of EDHF means it is inhibited either by increasing the concentration of extracellular potassium ions or by the presence of potassium channel inhibitors. As a result, the ensuing smooth muscle relaxation responsible for vasodilatation is blocked. In general, it seems that the potassium channels responsible for this are the intermediate and small conductance calcium-activated channels (IK_{Ca} and SK_{Ca}, respectively), and that they are located on the endothelium (Busse *et al.*, 2002, Edwards *et al.*, 1998, Hashitani *et al.*, 1997; Crane *et al.*, 2003a). Activation of these channels is a key event, leading to an endothelial cell hyperpolarization which is transmitted to the smooth muscle through gap junctions and/or the release of a diffusible factor(s). The identity of EDHF is still controversial with many possibilities proposed, including diffusible factors like EETs (Fleming, 2004), potassium ions effluxing through IK_{Ca} and SK_{Ca} (Edwards *et al.*, 1998), and under some conditions hydrogen peroxide (Shimokawa and Matoba, 2004). In addition to these diffusible factors, the heterocellular coupling between endothelial and smooth muscle cells, which allows the passage of hyperpolarizing current, has also been proposed as an EDHF (Yamamoto *et al.*, 1999; Imaeda *et al.*, 2000; Yamamoto *et al.*, 2001; Mather *et al.*, 2005). But whatever the precise signal in any particular vessel or region, the initiating event is an increase in cytoplasmic Ca^{2+} concentration stimulated by endothelium dependent vasodilators such as ACh (Fukuta *et al.*, 1999; McSherry *et al.*, 2005).

Mechanisms involved in spreading vasodilatation

When considering the phenomenon of spreading vasodilatation, a key question is whether or not the EDHF response observed at the site of application of the agonist, termed the 'local' response, may also be conducted upstream to become responsible for the vasodilatation observed at distal sites, *i.e.* the 'spreading' response. In this respect, a key observation was that spread was only evoked by dilator agents that were able to cause hyperpolarization (Delashaw *et al.*, 1991). This included ACh, which is widely used as an endothelium-dependent agonist in studies of both spreading vasodilatation and EDHF responses. In the former, ACh is applied focally, usually with a pressure-ejection micropipette, to an isolated and pressurized artery/arteriole with an intact endothelium. In this way focal application of ACh to a downstream site can dilate the whole length of an isolated artery, with only a slight decay of vasodilatation with distance upstream from the localized point of agonist application (Emerson *et al.*, 2000; Dora *et al.*, 2003b; Takano *et al.*, 2004; Goto *et al.*, 2004). This spreading vasodilatation was capable of traveling over a distance of about 2 mm. The mechanisms responsible for this spreading vasomotor response have been investigated and are discussed below.

One possibility is that intraluminal flow causes or helps vasodilatation to spread. Flow-dependent vasodilatation was observed in association with increased shear stress, which elicits NO production in endothelial cells (Joannides *et al.*, 1995; Thorsgaard *et al.*, 2003). This potential mechanism to dilate arteries at remote sites is important in regulating blood flow, but cannot explain spreading vasodilatation, as conducted responses can be observed when NO synthesis is inhibited by an NO synthase inhibitor (Emerson *et al.*, 2000; Dora *et al.*, 2003b; Takano *et al.*, 2004; Goto *et al.*, 2004).

Another possibility is that perivascular nerves are involved. To test this hypothesis, the selective and potent sodium channel blocker tetrodotoxin (TTX) was used to inhibit nerve conduction. In the presence of TTX, neither the spreading vasodilatation induced by ACh, nor even the vasoconstriction induced by norepinephrine, were modified (Segal *et al.*, 1989). When electrical field stimulation was used to evoke spreading vasoconstriction or spreading vasodilatation, TTX abolished the spread of vasoconstriction but not of vasodilatation (Emerson *et al.*, 2001). Thus it would appear that perivascular nerves are not important in spreading vasodilatation.

As endothelial cell Ca^{2+} is a fundamental factor in causing the hyperpolarization which leads to local vasodilation, is it involved in spreading vasodilatation?

Gap junctions allow small molecules such as Ca^{2+} and IP_3 to pass to adjacent cells (Dora, 2001a; Burdyga *et al.*, 2003). But, while ACh did stimulate a rise in Ca^{2+} concentration in the endothelial cell at the local site of application, no increase was observed within a distance of 0.5 mm upstream of the stimulating pipette (Dora *et al.*, 2003b; Takano *et al.*, 2004) while vasodilatation spread over the entire segment of isolated artery, for a distance of about 2 mm. So the rate or magnitude of spread of Ca^{2+} between cells is not sufficient to explain the relatively massive spreading response.

ACh evoked a spread of hyperpolarization in association with spreading vasodilatation (Emerson *et al.*, 2000; Crane *et al.*, 2004; Takano *et al.*, 2004). Injecting hyperpolarizing current

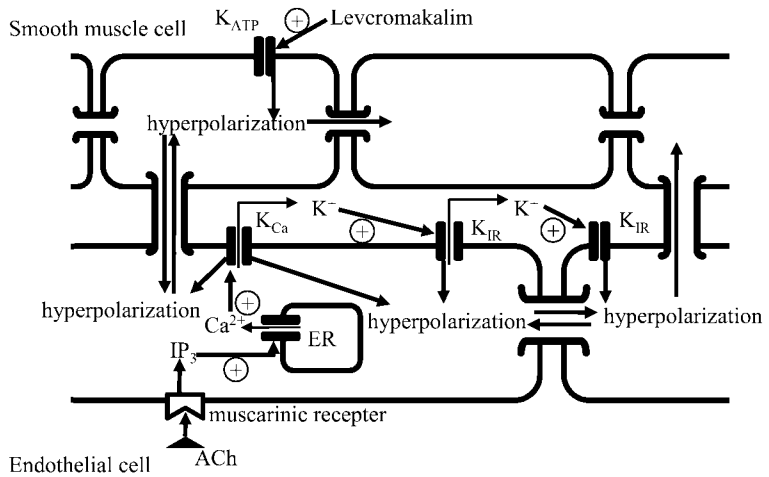

Fig. 1. The mechanisms of spreading hyperpolarization. ACh activates muscarinic receptors on the endothelial cell membrane and produces IP_3 which then activates IP_3 receptor channels on the endoplasmic reticulum. This causes the release of Ca^{2+} from the endoplasmic reticulum which then hyperpolarizes the endothelial cell membrane by activating Ca^{2+} activated K^+ channels. This hyperpolarization conducts to adjacent endothelial cells and smooth muscle cells. ATP sensitive K^+ channels activated by levcromakalim on smooth muscle cells hyperpolarize the membrane and the hyperpolarization conducts to adjacent endothelial cells and smooth muscle cells. Inward rectifier K^+ channels are activated by K^+ which is released by any of the K^+ channels and thus may contribute to enhancement of the spreading hyperpolarization. ER: endoplasmic reticulum. K_{Ca}: Ca^{2+} activated K^+ channel. K_{IR}: inward rectifier K^+ channel. K_{ATP}: ATP sensitive K^+ channel.

into an endothelial cell also induced spreading vasodilation (Emerson *et al.*, 2001). In addition, in resistance arteries, smooth muscle hyperpolarization occurred at distant upstream sites after focal ACh application (Takano *et al.*, 2004). Thus coordinated vasodilatation along the long axis of arterioles and arteries seems to involve concomitant spread of hyperpolarizing current (Fig. 1). This hypothesis is also supported by the fact that dilatation evoked by application of a NO donor, which did not itself cause smooth muscle hyperpolarization, failed to spread (Hoepfl *et al.*, 2002).

The endothelium seems to play an additional important role in the spreading responses. When the endothelium is damaged, the spreading vasodilatation is blocked (Takano *et al.*, 2004; Goto *et al.*, 2004). Levcromakalim, which activates ATP-sensitive K^+ channels causing smooth muscle cell hyperpolarization, could also elicit spreading vasodilatation and hyperpolarization (Fig. 1), but without any detectable change in intracellular concentration of Ca^{2+} in the endothelium in the rat mesenteric artery (Takano *et al.*, 2004). In this artery, the ATP-sensitive K^+ channels are restricted to the smooth muscle layers (White *et al.*, 2000; Takano *et al.*, 2004). Both spreading vasodilatation and hyperpolarization in response to levcromakalim were significantly inhibited in an endothelium denuded artery, in spite of the observation that the local hyperpolarization to this agent was unaltered. So, hyperpolarization elicited in a smooth muscle cell is conducted to adjacent endothelial cells and then passes through the endothelial

layer to remote sites to hyperpolarize and relax the smooth muscle cells, leading to vasodilatation. The primary importance of the endothelium in enabling spreading responses is supported by measurements of electrotonic potentials in guinea-pig arterioles (Yamamoto *et al.*, 2001). When current was injected into an endothelial cell, electrotonic potentials could be detected in another endothelial cell or in smooth muscle cells, and without any decay up to a distance of 350 μm from the injected cell. In contrast, the passive spread of current injected into a smooth muscle cell did not pass to other smooth muscle cells unless the endothelial cell layer was intact.

If hyperpolarization is the crucial element required to elicit the spreading responses, it is interesting that the distance over which spreading vasodilation occurs is much longer than the length which cable theory predicts. In arterioles, the space constant for decay of injected current is only about 1 mm (Hirst *et al.*, 1978). It is of interest that the length constants observed during vascular stimulation with ACh were larger than those observed with current injection alone (Emerson *et al.*, 2002). Thus, in some way, the spreading vasodilatation elicited by ACh is associated with facilitation of the spread of hyperpolarization (Crane *et al.*, 2004).

One possible explanation is that activation of inward rectifier K^+ channels (K_{IR}) in the artery may underlie facilitation (Rivers *et al.*, 2001; Horiuchi *et al.*, 2002) (Fig. 1). In this context, it is interesting that in the rat mesenteric artery, K_{IR} channels seem to be restricted to the endothelium (Dora *et al.*, 2001b; Crane *et al.*, 2003b).

Conclusion

Spread of vasodilatation over long distances has been observed in small resistance arteries, as well as in arterioles which only have a single smooth muscle cell layer, and in both cases these have strong electrical coupling existing between their smooth muscle cells and the endothelium. The underlying mechanism for the spread of dilatation appears to be a passive electrotonic spread of hyperpolarization. The endothelial cell layer appears to play an important role in this spread, but the fact that the predicted space constant is much less than the actual spread indicates some facilitatory mechanism must participate, particularly in the resistance arteries where the increasing number of smooth muscle layers would be expected to dissipate current rapidly.

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