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Local, integrated control of blood flow Professor Tudor Griffith Memorial

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ABSTRACT

Professor Tudor Griffith was one of the founding members of the European Study Group on Cardiovascular Oscillations, and hosted the 1st ESGCO Conference in Cardiff, Wales in 2000. Tudor was a passionate scientist, who managed to combine his enthusiasm for vascular biology with his background in physics, to make key and insightful advances to our knowledge and understanding of the integrated vascular control mechanisms that co-ordinate blood flow in tissue perfusion. He had a particular interest in the endothelium, the monolayer of cells that lines the entire cardiovascular system and which is in prime position to sense a wide variety of modulatory stimuli, both chemical and mechanical.

Over the last 20 years Tudor produced a series of research papers in which he used chaos theory to analyse the behaviour of arteries that underpins vasomotion. The research led to the development of mathematical models that were able to predict calcium oscillations in vascular smooth muscle with a view to predicting events in a complete virtual artery.

This article will review the field in which he worked, with *an obvious* emphasis on his contribution.

1. Vasomotor control

1.1. Local regulation

Under basal conditions arterial smooth muscle exhibits some degree of contraction, a feature that determines the tone and diameter of the vessel; in the absence of such tone cardiac output would be insufficient to maintain the circulation (Bevan and Laher, 1991). Additionally a level of basal tone enables a vessel to respond to stimuli, to vasodilate allowing increased localised blood flow and tissue perfusion, such as that required by increased metabolic demand. Vasodilation can be considered to be a loss of tone, as it is a function that is reliant on basal vascular tone which varies between vessel type, dependent on the requirements of the tissue or organ being perfused by the vessel. Vascular tone is controlled by a balance between competing vasoconstrictor and vasodilator influences determined by a variety of extrinsic and intrinsic control mechanisms.

Extrinsic factors originate from outside the tissue in which the blood vessel is located. Examples of extrinsic control mechanisms include circulatory hormones such as angiotensin II, and neuromodulatory control by neurotransmitters such as catecholamine released from sympathetic perivascular nerves and acting through adrenoceptors on vascular smooth muscle cells (Burnstock, 1993). Intrinsic factors originate within the blood vessel and include those inherent to smooth muscle cells (myogenic mechanisms), factors released by the endothelium and from the surrounding tissue, (such as by-products of tissue metabolism and other biochemical pathways). Many organs thus have an innate ability to influence and maintain their own blood supply via intrinsic factors, a mechanism termed “local regulation”. The intrinsic mechanisms responsible for local regulation act independently of extrinsic control, and can therefore be demonstrated in isolated organs, a situation in which there is no neural or hormonal influence.

1.2. Vascular smooth muscle contraction, myogenic tone

The mechanisms by which intrinsic factors influence blood vessel tone involve a variety of signal transduction mechanisms, all of which ultimately influence the regulation of cytosolic free Ca^{2+} $[Ca^{2+}]_i$ within the smooth muscle, and activation of the contractile machinery. Calcium combines with calmodulin and the complex formed interacts with myosin light chain kinase (MLCK) an enzyme that phosphorylates myosin light chain, thereby permitting interactions with actin and triggering the cycling of myosin crossbridges along the actin filaments with the development of force (Hill et al., 2001).

The chain of processes linking a stimulus to the contractile response of a muscle is known as excitation–contraction coupling (EC) and two major types have been described in vascular smooth muscle, namely electromechanical and pharmacomechanical. Fluctuations in $[Ca^{2+}]_i$ during electromechanical coupling depend on changes in the membrane potential of the cell. Depolarization of the plasma membrane induces the opening of voltage operated (L-type) Ca^{2+} channels (VOCs), allowing the influx of Ca^{2+}. Hyperpolarization, predominantly through the opening of potassium channels, closes VOCs. Pharmacomechanical

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coupling is initiated through receptor occupation by agonists promoting activation of plasmalemmal receptor operated Ca^{2+} channels, or generation of the second messenger inositol 1,4,5-trisphosphate ($InsP_3$) and Ca^{2+} release from the main intracellular Ca^{2+} store, the sarcoplasmic reticulum (SR), via stimulation of the $InsP_3$ receptor (Guibert et al., 2008). Ca^{2+} release from the SR is also mediated through the mechanism of Ca^{2+} induced Ca^{2+} release (CICR) via ryanodine sensitive Ca^{2+} release channels (RyR) (Hill-Eubanks et al., 2011).

1.3. Myogenic mechanisms

Smooth muscle cells are effectively in a continuous state of activation and this is partially due to the myogenic response, a mechanism that originates in the smooth muscle of blood vessels and is particularly pronounced in small arteries and arterioles. When the lumen of a blood vessel is suddenly expanded, as occurs when intravascular pressure is suddenly increased, the tension in the wall of the vessel also increases, this leads to Ca^{2+} entry through stretch activated voltage-dependent and -independent mechanisms and activation of the contractile proteins, this is the myogenic response (Hill et al., 2001). These events are likely supported by a number of other mechanisms, including the rearrangement of cytoskeletal elements, Ca^{2+} release from the SR and increased Ca^{2+} sensitivity of the contractile elements. The myogenic response can be regarded as unstable positive feedback and can spread to adjacent segments of the same vascular network with the potential for uncontrolled vasoconstriction and instability in the intact circulation. Negative feedback is provided in smooth muscle by the rise in Ca^{2+}, promoting Ca^{2+}-sensitive potassium channel (K_{Ca}) activation leading to hyperpolarization, and through cross talk with the endothelium (Hill et al., 2001).

1.4. Endothelium and autoregulation

In 1980 Furchgott and Zawadzki established the endothelium as a key modulating influence on vasodilatation. They discovered that stimulation of muscarinic receptors on endothelial cells stimulated the release of a mysterious endothelium-derived relaxing factor (EDRF), that caused potent relaxation of vascular smooth muscle. EDRF was shown to be labile diffusible factor, with a half-life of around 6 s (Griffith et al., 1984), with its production dependent on extracellular calcium (Griffith et al., 1986). The vascular smooth muscle relaxation by EDRF was predominantly through activation of the enzyme soluble guanylate cyclase and the formation of the second messenger cyclic nucleotide cyclic guanosine monophosphate (cGMP) (Rapoport and Murad, 1983; Griffith et al., 1985). cGMP-mediated effects include, Ca^{2+} desensitization of myosin activity (Somlyo and Somlyo, 2003) and a reduction in the $[Ca^{2+}]_i$ available for contraction through a number of mechanisms (Griffith, 1994; Francis et al., 2010). In 1987 it was established that EDRF was nitric oxide (NO) (Palmer et al., 1987), formed in the endothelium from its precursor L-arginine, by the calcium sensitive constitutive enzyme endothelial Nitric Oxide Synthase (eNOS) (Bredt and Snyder, 1990).

The agonist stimulated rise in endothelial $[Ca^{2+}]_i$ required to activate eNOS is biphasic. An initial rise in $[Ca^{2+}]_i$ from $InsP_3$-sensitive endoplasmic reticulum (ER) stores, is followed by a sustained elevation mediated by Ca^{2+} influx through activation of the capacitative Ca^{2+} entry pathway that follows depletion of the ER Ca^{2+} store (Sedova et al., 2000). In addition to pharmacological stimulation, NO is continuously released in the basal state (Griffith et al., 1987a), and the endothelium responds to hemodynamic forces such as shear stress and stretch and release of NO is sensitive to flow velocity, viscosity and pulsatility (Hutcheson and Griffith, 1991). Shear forces are sensed by multiple mechanotransducer molecules, the cytoskeleton and membrane components that transmit the signal into the interior of the endothelial cells. The event triggers a variety of cellular responses through stretch-activated channels and activation of G proteins that elevate $[Ca^{2+}]_i$ within seconds (Hutcheson and Griffith, 1997; Balligand et al., 2009). In addition to being a potent vasodilator NO also has other actions on the vasculature. NO: has antithrombotic actions, inhibits platelet aggregation and leucocyte adhesion and penetration; prevents proliferation of vascular smooth muscle cells; prevents the formation of oxidised low-density lipoprotein cholesterol (LDL) (Moncada et al., 1991, Vanhoutte et al., 2009). Consequently, a reduction in bioavailability of NO, as in the case of increased reaction rates with the superoxide anion under conditions of oxidative stress, leads to pathological disorders such as atherosclerosis (Napoli et al., 2006).

1.5. NO and flow in vascular networks

Griffith and colleagues developed techniques using X-ray microangiography that allowed simultaneous imaging of vessels of different sizes in an isolated, yet intact, vascular bed. The technique was especially suited to study responses to changes in flow in the presence and absence of NO activity (Griffith et al., 1987b, 1988; Randall and Griffith, 1993). NO-mediated dilatation to flow was shown to co-ordinate changes in calibre throughout the vascular bed, illustrating the interdependence of vessels and emphasising the need to consider integrated behaviour as a whole. The endothelium was shown to confer stability by superimposing an opposing feedback mechanism to the myogenic response within the smooth muscle, thus preserving constancy of flow distribution at different flow rates to prevent vascular "steal" (Griffith and Edwards, 1990a). Studies on the distribution of flow at arterial bifurcations suggested that, over a wide range of flow, basal NO activity maintained geometrical optimality in terms of minimum volume and power losses, helping to minimise cardiac work by allowing rapid changes in flow to occur with only small changes in central arterial pressure (Griffith and Edwards, 1990b).

Microcirculatory control should ultimately serve to match organ perfusion to metabolic requirements. Stabilizing mechanisms such as autoregulation, maintenance of flow distribution, and limitation of cardiac work relative to perfusion are important considerations for overall cardiovascular function. By modulating local vascular tone in response to the integrating signal of blood flow through NO, the endothelium couples changes in resistance in different parts of the vascular bed and thereby contributes to the co-ordination of all local control mechanisms. NO also participates in the regulation of flow through feedback loops that involve extrinsic factors such as neurogenic mechanisms of vascular regulation. Under conditions of sympathetically-mediated arterial constriction, shear forces will increase and the resultant enhancement of NO synthesis may exert inhibitory prejunctional effect on catecholamine release and diminish the original constrictor stimulus (see Griffith, 1994 for review). Thus any impairment of NO activity will have adverse effects on its important physiological role in maintaining "efficiency" of perfusion.

2. Vasomotion

Vascular smooth muscle is often observed to undergo "spontaneous" rhythmic contractile activity that leads to oscillations in vascular diameter, a phenomenon known as vasomotion (Pradhan and Chakravarthy, 2011). Vasomotion can be seen in many, if not all, vascular segments, it occurs both in vivo and in vitro, and is generated from within the vascular wall so that it is not a consequence of the heart beat, respiration or neuronal input (Aalkjaer and Nilsson, 2005). Although the physiological significance of vasomotion remains the subject of ongoing debate, it is widely assumed that the local fluctuations in tissue perfusion confer an advantage over steady-state flow. At the simplest functional level, by continuously redistributing flow, vasomotion will ensure that all tissue elements ultimately receive perfusion. Indeed, in pathological states such as haemorrhagic shock, vasomotion becomes particularly

pronounced and may then represent an adaptive homeodynamic response that helps to preserve perfusion and limit tissue damage. More subtle effects of vasomotion may be to limit extravascular fluid filtration by reducing hydrostatic pressure during periods of low flow, and to enhance lymphatic drainage through a 'pumping' action by causing periodic compression of lymphatics that encircle arterioles and whose valves will prevent retrograde flow (see Griffith, 1996 for review).

2.1. Chaos

By analysing fluctuations in tension, pressure, flow and diameter observed in perfused or isometrically mounted segments of isolated arteries, Griffith and colleagues studied intrinsic local mechanisms involved in the genesis of vasomotion. In these isolated preparations vasomotion occasionally arose spontaneously, but in general rhythmic activity appeared on a more consistent basis when the artery was exposed to constrictor agonists such as histamine or phenylephrine. The vasomotion usually appeared irregular and thus superficially "random", but often exhibited highly distinctive patterns of behaviour that may be considered "universal" in the sense that they may also be observed in non-biological systems and nonlinear mathematical models (Griffith and Edwards, 1994a). A key characteristic of nonlinear systems is that they can readily switch between different operational modes, e.g. from stable steady states to periodic behaviour or non-repetitive patterns of activity that can be classified as "chaotic". Inspection of the experimental data revealed well-recognized routes to chaos including exactly even- or odd-periodic activity, quasiperiodicity and intermittency (Griffith and Edwards, 1994a). Such observations provided evidence that rather than simply representing "noise" generated by stochastic events vasomotion, in these isolated arteries, is deterministic in origin, can be classified as 'chaotic' and can therefore be analysed within the framework of nonlinear dynamical systems theory.

Non-linear mathematical analysis allows estimation of the phase space dimension of chaotic signals obtained by continuous observation of a single experimental parameter, even when the underlying control variables may be unknown. As chaotic trajectories do not occupy the phase space available to them completely, such estimates are generally non-integral and may therefore be considered as a fractional (or 'fractal') dimension. The most commonly employed method is by Grassberger and Procaccia (1983), in which the fractal dimension of an irregular time series is calculated as a correlation dimension (D_c). The algorithm identifies the lowest possible number of contributing variables, when rounded to the nearest upper integer. For the irregular oscillations in pressure and flow, time series were digitized as a series of temporally equidistant points with 30–40 points per average oscillatory cycle and a total of at least 1000 data points used for calculation. The vasomotion, produced in the isolated arteries, generally gave values of D_c in the range 2 to 4, a result that is consistent with classification as low-dimensional chaos and suggests that just four dynamic variables could account for the patterns of rhythmic activity observed experimentally (Griffith and Edwards, 1994a).

Changes in the concentration of histamine used to induce irregular activity, altered perfusion pressures and exerted complex effects on the superficial appearances and amplitude of the vasomotion. However, fractal analysis showed that such interventions did not affect the intrinsic mathematical complexity of the time series (Griffith and Edwards, 1994a). Stimulation of NO activity superficially suppressed vasomotion, however fractal analysis showed that either inhibition or stimulation of endothelial NO synthesis had no fundamental effect on its intrinsic mathematical complexity. These findings suggested that NO is a modulatory factor rather than a primary control variable in the genesis of vasomotion (Griffith and Edwards, 1994a). This is an interesting observation since the influence of the endothelium for vasomotion seems to vary between different vascular beds. In some artery types, blockade of NO production or removal of the endothelium prevents vasomotion, whereas in others vasomotion is promoted when eNOS is inhibited or the endothelium is removed (Aalkjaer and Nilsson, 2005). The highly unpredictable responses of non-linear systems to perturbation, rather than fundamental differences in smooth muscle control mechanisms, may explain why NO can either suppress or enhance rhythmic vasomotor activity in different artery types, depending on initial conditions and Ca^{2+} homeostasis. Changes in flow rate (and again perfusion pressure) also markedly influence the oscillatory patterns observed, but did not alter the dynamical complexity of the responses in a given preparation. Thus, flow and pressure are unlikely to be primary determinants of the complexity of vascular vasomotion under physiological conditions, and should not be considered as key control factors (Griffith and Edwards, 1995; Griffith, 1996).

2.2. Control mechanisms

Some experimental pressure traces from isolated vessels showed clear evidence for the quasiperiodic route to chaos. Quasiperiodicity consists of motion governed by several principal frequencies, and power spectra from a number of biperiodic pressure traces confirm the existence of 2 principal frequency components with an obvious slow (period 1–5 min) and fast (period 5–20 s) oscillatory component (Griffith and Edwards, 1994a, 1994b). As stated above, the principal determinant of smooth muscle contraction is $[Ca^{2+}]_i$, regulated by coupled intracellular and membrane subsystems that involve Ca^{2+} uptake and release from internal stores and influx and efflux of Ca^{2+} across the cell membrane. A series of pharmacological experiments in isolated arteries provided experimental evidence that universal behaviour and chaos both result from nonlinear interactions in the smooth muscle cell between an intracellular oscillator involving Ca^{2+}-induced Ca^{2+} release from intracellular stores, and a membrane oscillator involving transmembrane Ca^{2+} and K^+ fluxes (Griffith and Edwards, 1994b, 1997).

Confirmation that the slow intracellular subsystem of vasomotion is dependent on cyclic uptake and release of Ca^{2+} from internal stores was obtained with ryanodine, which inhibits a specific Ca^{2+}-sensitive Ca^{2+} release channel on the sarcoplasmic reticulum (SR) of vascular smooth muscle. Ryanodine selectively abolished the slow, but not the fast, component of the vasomotion (confirmed by spectral analysis), without affecting mean perfusion pressure, simplifying the patterns of vasomotion observed experimentally and reducing estimates of D_c by one or more units (Griffith and Edwards, 1994b). The fast subsystem was dependent on Ca^{2+} influx across the cell membrane and could be selectively inhibited either by a reduction in the extracellular Ca^{2+} concentration, or blockade of individual components of a multiple coupled transmembrane ion transport system, that promote membrane hyperpolarization, including voltage-operated Ca^{2+} channels, K_{Ca} channels and the Na^+–K^+ ATPase (Griffith and Edwards, 1997). Entrainment of the ion channels leads to the emergence of a composite membrane oscillator, thus accounting for the low fractal dimension of the vasomotion observed in these arteries. As in the case of ryanodine, blocking the participating ion channels produced a concentration-dependent fall in the average value of D_c, from between 2 and 3 to <2 (Edwards and Griffith, 1997).

The nonlinear crosstalk between plasmalemmal and SR oscillatory subsystems resulted in the patterns of universal behaviour and chaos observed and also leads to apparently paradoxical behaviour where experimental administration of the Ca^{2+} antagonist verapamil or changes in flow rate may either induce large-amplitude vasomotion in some artery preparations, or completely suppress rhythmic activity in others (Griffith and Edwards, 1994b, 1995).

3. Modelling

To investigate whether chaotic activity may contribute to the regulation of flow in ways not possible with periodic behaviour, vasomotion was simulated using a theoretical model of flow. Sinusoidal and chaotic

diameter fluctuations were employed in simulations, which were validated against experimentally observed oscillations in flow and pressure. The model employed suggested that vasomotion may serve to increase time-averaged flow, and that chaotic vasomotion dissipates transients more readily than sinusoidal vasomotion, thereby conferring greater stability to microcirculatory perfusion (Parthimos et al., 1996).

The experimental findings, with pharmacological tools on isolated arteries, allowed the formulation of a novel 4-dimensional mathematical model of Ca^{2+} movements in the smooth muscle cell. The model is based on the coupling between the cytosolic and membrane subsystems, which has been shown to be nonlinear (De Brouwer et al., 1998). In theory each subsystem requires two distinct control variables to generate oscillatory activity. The control variables chosen for the model were $[Ca^{2+}]_i$, $[Ca^{2+}]$ in intracellular stores, membrane potential and K_{Ca} open state probability. By varying the weights of different ion transport systems the model can be made to generate highly realistic simulations of the patterns of chaotic behaviour observed experimentally, and well-recognized "routes to chaos" such as quasiperiodicity, intermittency, and "mixed-mode response". The model was also able to explain the apparently paradoxical effects of pharmacological interventions, such as the observation that Ca^{2+} channel antagonists may either promote or eliminate vasomotion entirely (Parthimos et al., 1999).

For the 4-dimensional model, regenerative intracellular Ca^{2+}-induced Ca^{2+} release was assumed to be mediated exclusively via the ryanodine receptor (RyR), and a coexisting $InsP_3$-sensitive store was assumed to provide a steady flux of Ca^{2+}into the cytosol. Parthimos et al. (2007) developed a 3-dimensional model of vasomotion employing $[Ca^{2+}]_i$, $[Ca^{2+}]$ in intracellular stores and membrane potential as dominant variables, but in which a second Ca^{2+}-induced Ca^{2+}release mechanism via the $InsP_3R$ was incorporated. This approach allowed evaluation of the dynamical consequences of differences in the kinetics of the RyR and $InsP_3R$ and interactions between the two channels could be studied by varying their relative contributions to intracellular Ca^{2+}movements. The 3-dimensional model was sufficient to reproduce many of the relatively simple patterns of vasomotion observed experimentally, but was not able to produce the more complex nonlinear behaviour reproduced by the four variable model (Parthimos et al., 2007).

Both models assume that the macroscopic activity of an artery, observed as vasomotion, reflects oscillations in $[Ca^{2+}]_i$ in individual smooth muscle cells, that synchronize their activity in the vessel wall. Therefore, it should be noted, that by coupling nearly identical limit-cycle oscillators, such as that generated by the three-variable model, it is possible to produce more complex chaotic behaviour. Studies are therefore necessary to compare the advantages of a coupled-cell approach rather than a single high-order system of differential equations to model vasomotion. Although it is well-established that the synchronisation of Ca^{2+} oscillations in vascular smooth muscle cells is dependent on gap junctional communication (see below), it remains unclear whether the dominant intercellular coupling mechanism is electrical or chemical in nature. For completeness, further modelling studies would therefore need to incorporate the cytoplasmic distribution of stores expressing the RyR and the $InsP_3R$, intracellular and intercellular diffusion of Ca^{2+} ions and $InsP_3$, as well as electrical coupling between adjacent cells. A more complex, compartmentalized model would also allow delineation of how stores located close to the cell membrane might target and activate channels in a spatially selective fashion. Such studies are likely to suggest experimental approaches that can be tested pharmacologically and thereby clarify the signalling pathways that lead to emergent behaviour.

4. Gap junctions

The onset of "macroscopic" vasomotion, following the administration of constrictor agonists, coincides with the unidirectional synchronisation of intracellular Ca^{2+} waves that normally, under resting conditions, propagate bidirectionally through the cytosol of individual smooth muscle cells (Peng et al., 2001). Such entrainment is dependent on direct coupling via gap junctions since pharmacological interruption of direct cell–cell communication suppresses the synchronisation of Ca^{2+} oscillations in both intact arteries and cultured smooth muscle cells (Martin et al., 2005; Matchkov et al., 2006). Gap junctions are composed from two hemichannels, whose docking across the extracellular space permits intercellular diffusion of ions and small molecules 1 kDa in size and confers electrical continuity, that allows the relay of signals over biologically significant distances almost without decrement (see de Wit and Griffith, 2010 for review). Homocellular signalling between endothelial or smooth muscle cells may contribute to arterial function by permitting local dilations/constrictions to propagate longitudinally along the vessel wall. Pacemaker sites may therefore initiate the propagation of active vasomotor responses, so that rhythmic changes in diameter at any given site in the microcirculation in vivo may in theory reflect the resultant conducted upstream and downstream activity superimposed on local vasomotion (de Wit and Griffith, 2010).

Heterocellular gap junctions allow electrical and/or chemical coupling between vascular smooth muscle and the endothelium. Electrotonic spread of endothelial hyperpolarization, to the vascular smooth muscle, plays a critical role in a NO-independent, endothelium-dependent, mechanism for the control of arterial tone (the "EDHF-phenomenon", see Griffith, 2004 for review). This endothelium-dependent hyperpolarization mechanism (EDH, see Félétou and Vanhoutte, 2013) is initiated by endothelial hyperpolarization, dependent on the opening of K_{Ca} channels (Doughty et al., 1999), with subsequent hyperpolarization of the arterial media following electrotonic relay of this change in membrane potential via myoendothelial and homocellular smooth muscle gap junctions (Chaytor et al., 2005). Endothelial Ca^{2+} mobilization and hyperpolarization can also be modulated by hydrogen peroxide (H_2O_2) suggesting that endothelial redox signaling may play a novel role in the EDHF phenomenon under normal and pathophysiological conditions (Edwards et al., 2008). If so, the increased endothelial oxidant stress and H_2O_2 production from superoxide anions that is a feature of diseases such as hypertension, hypercholesterolemia, and diabetes, might offset reductions in the bioavailability of NO resulting from its direct chemical interaction with superoxide (see de Wit and Griffith, 2010 for review).

5. Future studies

The natural progression is to take the mathematical modelling of calcium oscillations in the vascular wall forward, to eventually evolve as a complete virtual artery. The model would need to include multicellular arterial architectures that take into account the important interaction between the smooth muscle and endothelial cell types. Tudor Griffith believed that combined theoretical/experimental studies would provide novel insights into the role of nonlinear control mechanisms in the vessel wall, thereby defining key biological questions. An approach that has the potential to facilitate the rational design of new strategies for the pharmacological manipulation of vascular function.

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