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The use of excised, pressurized blood vessels to study the physiology of vascular smooth muscle

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Introduction

Many techniques of using excised blood vessels have been utilized to obtain information on the physiology, pharmacology and pathophysiology of vascular smooth muscle[4]. Surprisingly, the most physiological of these preparations, pressurized blood vessels, has been little used other than for the study of the mechanical properties of maximally activated arteries[6,8]. Two important reasons for this relative neglect have been the unavailability of simple methods for measuring vessel diameter and, secondly, the complex relationship between the recorded perfusion pressure, the rate of perfusion, the geometry of tubes (Poiseuille's formula) and the active stress-muscle length characteristics of the smooth muscle. Many techniques are now available for accurately and continuously recording the vessel diameter. These vary from elegantly simple (and inexpensive) image sensing[12] to the sophisticated video-electronic and video-optical techniques needed to measure the diameter of microvessels[2,9]. Moreover, interpretational complexities can be simplified by using unperfused but pressurized vessels[6,14], or by inserting a variable, mechanical outflow resistance and adjusting a constant rate of perfusion to minimize the pressure gradient along the artery[1,13]. The availability of these techniques is relevant as there are reasons for suspecting that the widespread use of rings and strips of blood vessels has led to the neglect of important physiological properties of vascular smooth muscle. The geometry of tubes imposes functional requirements on the muscle present in the wall which need to be fulfilled. The purpose of this article is to outline some of these functional requirements, to indicate ways in which they may be met and to discuss briefly the future use of single, pressurized blood vessels.

Functional requirements imposed upon vascular muscle by the geometry of tubes

Figure 1 shows the calculated changes in stress (force/wall cross-sectional area) on the muscle of perfused ear arteries of the rabbit when they were made to constrict against a constant transmural pressure[16]. Under these conditions, muscle contraction is not isotonic as the applied force first increased and then decreased with increasing constriction. Consequently, considerable active stress was needed to produce a modest constriction. Less active stress was required for greater constrictions which predisposes towards further constriction. This inherent positive feedback was recognized by Burton[5] and formed the basis of his hypothesis of critical closing pressure. Blood vessels therefore need to have, as a consequence of the geometry of tubes, some mechanism which both overcomes the dimensional instability and enables the muscle to develop the high active force required for a modest constriction. Such a mechanism may need to operate with some precision as blood flow is a high power function of the internal radius of a blood vessel. Precise control may be particularly important for those vessels which play a major role in regulating the peripheral resistance or have the capacity to do so. Folkow[10] believes that the arterial vessels most involved in regulating the peripheral resistance are those with relaxed internal radii of more than 40–50 μm. The larger the resistance vessel, the more crucial control becomes. Some muscular arteries such as the central ear artery of the rabbit and coronary arteries in variant angina have the capacity to shut off or severely restrict the blood supply to much of an organ. Any blood vessel may be used to examine the effect of tube geometry on vascular muscle behavior, but it is desirable that the selected vessel be free of side branches (or, if present, can be tied off), is of sufficient length so that both ends of the segment can be cannulated without compromising the function of the remainder of the artery, has minimal taper and is muscular.

Fulfillment of the functional requirements

The closed circles (fig. 1) show the steady-state constriction achieved when initially relaxed arteries were made to

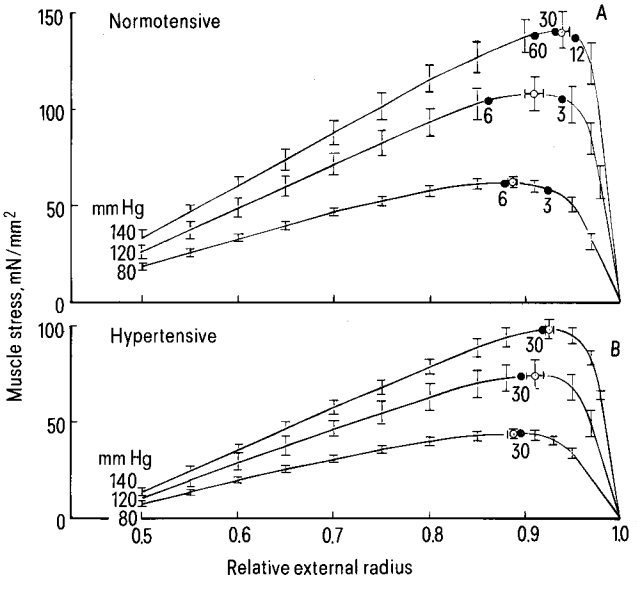

Figure 1. Changes in stress on the muscle of perfused ear arteries of the rabbit during constriction against a constant transmural pressure. The stress (force/wall cross-sectional area) was obtained by subtracting the passive circumferential from the total circumferential wall stress. The closed circles (●) show the muscle stress at completion of constriction. The perfusate concentration (nmol dm^{-3}) is given beside each closed circle. The arteries were obtained from normotensive (A) and renal hypertensive (B) rabbits. From Speden and Ryan[16].

constrict against a constant transmural pressure with different perfusate concentrations of noradrenaline. Weak concentrations of noradrenaline did produce the high active force needed for a modest constriction and more constricted arteries were dimensionally stable. There are at least two factors which may contribute to this fulfillment of the functional requirements imposed by the geometry of tubes. One is the position of the muscle cells on their active stress-cell length curves, as the active force of maximally activated arteries increases with distension up to a maximum and then declines[6]. These changes in active wall stress with distension favor force development when the arteries are distended and tend to counteract the inherent dimensional instability of blood vessels. The decreasing stress on the muscle with increasing constriction may be partially offset by a simultaneous decline in active wall stress. The other known factor, with active arteries, is stretch-activation of the muscle. Perfused ear arteries of the rabbit constricted equally well against transmural pressures of 80 mm Hg and 120 mm Hg, although the stress on the muscle was nearly twice as great at the higher pressure (fig. 1A). They did so because the active artery reacted to the distension produced by a jump in pressure by constricting[14, 15]. The dilatation produced was largely overcome within 1–2 min (fig. 2). This negative feedback reaction, which was reproducible for many hours, appears to be a property of the intact, pressurized blood vessel as no comparable reaction has been seen with strips or rings of arteries[14]. This reaction of active arteries to distension is not unique to the rabbit ear artery as perfused renal arteries from four mammalian species also showed a high resistance to distension[15]. Nor is it apparently restricted to muscular arteries as excised and pressurized arterioles (12–112 μm in diameter) showed a similar high resistance to distension when active[7, 9].

A negative feedback reaction which acts to minimize changes in blood vessel diameter, following changes in internal pressure, is an attractive alternative[14] to the Bayliss phenomenon[3] of enhanced constriction. The Bayliss phenomenon predisposes towards instability of the blood pressure[3], whereas a pressure-sensitive negative feedback reaction, located in the blood vessel wall, predisposes towards stability. The observed feedback reaction[14, 15] could provide the basis for stable control of the peripheral circulation by making blood vessel diameters relatively insensitive to normal fluctuations in blood pressure. More precise adjustments may then be achieved through other local and remote control systems such as accumulation or washout of metabolites[11] or sympathetic nerve impulses. A variety of local control mechanisms may well be operating simultaneously in vivo as the reactions seen with excised blood vessels[7, 9, 14, 15] cannot fully explain autoregulation of blood flow, although they may well contribute to it. Some additional local mechanism, or mechanisms, which enhances constriction of resistance vessels as the perfusion pressure rises needs to be present in vivo. Excised blood vessels, unlike terminal arterioles in situ[11], rarely reacted to increases in internal pressure with an enhanced constriction[14, 15].

Future use of excised, pressurized blood vessels

The above interpretation is speculative and many questions need to be answered before the physiological significance of the reaction of excised blood vessels to distension can be established. Blood vessels vary greatly anatomically, physiologically and pharmacologically[17] and

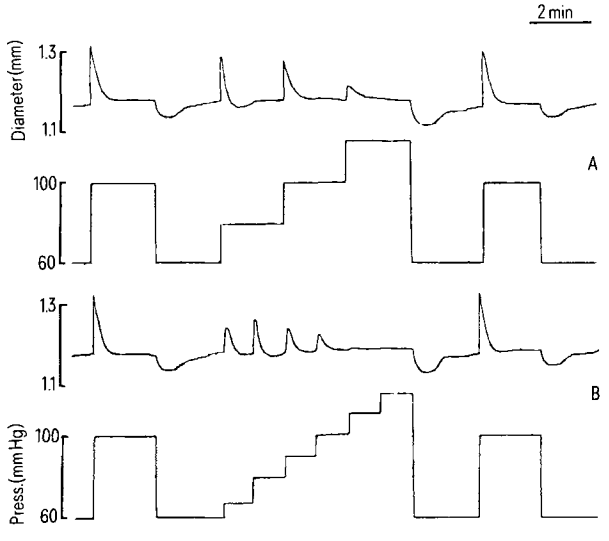

Figure 2. Reactions of a spontaneously active ear artery from a rabbit to jumps in transmural pressure. The external diameter (upper record in each panel) was recorded using a linear array of photodiodes. From Speden[14].

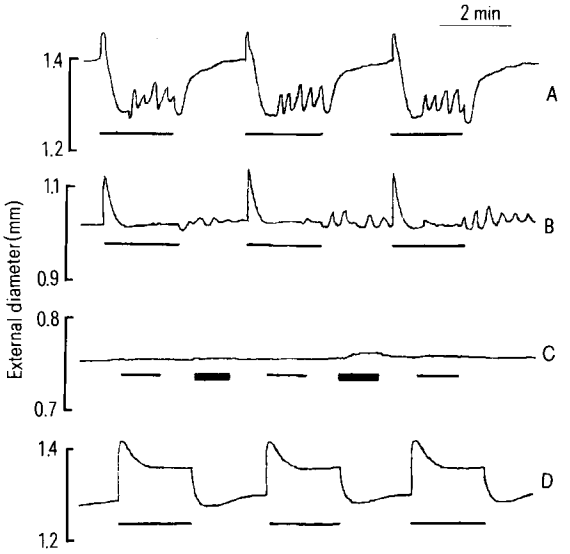

Figure 3. Effect of constriction on reactions of the same rabbit ear artery to jumps in transmural pressure. The transmural pressure was kept at 60 mm Hg except where indicated by the underlining when the pressure was jumped to 100 mm Hg (thin underlines) or to 160 mm Hg (thick underlines). Jumps in pressure enhanced constriction of the spontaneously active artery (A) which also became unstable. No enhancement was seen when the artery was constricted further with extraluminal noradrenaline (B), but the constriction did become unstable when the pressure was restored to 60 mm Hg. The maximally constricted artery was resistant to distension (C) and this maximal constriction impaired the reaction to distension (D). *D* was obtained 30 min after washing out the high concentration of noradrenaline (29.6 μmol dm^{-3}) used earlier (C). From Speden[15].

may fulfil the functional requirements imposed by the geometry of tubes to differing extents and in different ways. The reactions of a variety of excised, pressurized blood vessels need to be examined under many different experimental conditions, including pathophysiological conditions. Acute renal hypertension did not obviously disturb the negative feedback mechanism (fig. 1B), but this does not mean that the mechanism may not be upset in other pathophysiological situations.

The main advantage of pressurized blood vessels over rings and strips of these vessels is their potential ability to react to stretch and, under the appropriate conditions, to do so reproducibly. A pressurized, blood vessel preparation is more physiological in that the diameter is measured rather than calculated and the stresses on the wall (circumferential and axial) more closely approximate those in vivo[6]. Pressurized vessels may also be used to study other phenomena like phasic activity and spontaneous constriction (tone) which are rarely seen with strips or rings of blood vessels[18]. The pressurized ear artery of the rabbit may constrict spontaneously (figs 2 and 3) and the constriction can become unstable (fig. 3). Figure 3 also indicates that maximal activation needs to be used judiciously as it impairs active reactions to distension.

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Full Papers

Juvenile hormone titre and regulation in the cockroach *Diploptera punctata*

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Summary. Titres of juvenile hormone (JH) have been determined in both hemolymph and whole body extracts of female *Diploptera punctata* during the first gonotrophic cycle using a method employing gas chromatography/mass spectrometry for qualitative and quantitative analysis. JH III is the sole JH found in both adult and last instar *D. punctata*. Maximum values of ~ 1500 ng/ml (~ 6 μM) were observed at the middle of the gonotrophic cycle, when basal oocyte growth rate was greatest. Changes in rates of JH release in vitro by corpora allata paralleled closely the changes in JH titre, suggesting that biosynthesis is a major regulator of titre. JH levels per animal were calculated from observed JH titres, and at certain time points in the gonotrophic cycle JH levels obtained from analysis of whole bodies were significantly greater than those predicted from hemolymph titres. These results suggest the existence of a nonhemolymph JH pool in *D. punctata*.