APPS/PSNZ Meeting - Sydney 2003

Symposium 2: Potassium channels and endothelium-derived hyperpolarising factor: Physiological and clinical roles

Monday 29 September 2003

Chair: Caryl Hill

Endothelium-derived hyperpolarising factor and cell coupling: Factors and fiction?

S.L. Sandow, Division of Neuroscience, John Curtin School of Medical Research, ANU, Canberra, ACT 0200, Australia.

Together with nitric oxide and prostaglandins, endothelium-derived hyperpolarising factor (EDHF) is one of three vasodilatory factors produced by the arterial endothelium. The nature and mechanism of action of EDHF is the subject of intense current research interest. EDHF activity has been reported to be dependent on either the release of a diffusible substance from the endothelium or to the direct contact of endothelial cells and smooth muscle cells via gap junctions. Diffusible factors proposed as EDHFs include K^+ ions, H_2O_2, epoxyeicosatrienoic acids, L-NAME insensitive nitric oxide, and C-type natriuretic peptide. Contact-mediated EDHF is dependent on myoendothelial gap junctions (MEGJs) that enable the passage of small molecules, and/or direct electrical coupling between the two cell layers. In the latter case, this coupling would result in an endothelial cell hyperpolarisation being directly transferred to the smooth muscle, for the subsequent generation of an arterial relaxation. This latter mechanism represents the simplest explanation of EDHF activity.

Interestingly, it has been shown that the nature and mechanism of action of EDHF can differ along and between vascular beds, and that it can also change during development and in ageing and disease. Furthemore, in the mesenteric vascular bed of the rat, EDHF has been described to be K^+ ions, H_2O_2, L-NAME insensitive nitric oxide, CNP, as well as to be due to the direct electrical coupling of endothelial cells and smooth muscle cells. This variation is likely to be due to methodological differences between the laboratories in which such studies were made. Thus, the debate in the EDHF field is often clouded by such unfortunately inconsistent reports.

Studies from our laboratory have focused on the potential role of MEGJs in EDHF activity. We have found that the distribution and activity of MEGJs is correlated with the presence of EDHF within and between vascular beds, during development and in disease. In smaller distal mesenteric arteries of the rat, for example, MEGJs are more prevalent than in larger proximal vessels (Sandow & Hill, 2000), in line with the EDHF-mediated relaxation being more prominent in the smaller than in the larger vessels (Shimokawa *et al.*, 1996). In this vascular bed, EDHF-mediated hyperpolarisation and the transfer of endothelial cell hyperpolarisation are correlated with the presence of MEGJs (Sandow *et al.*, 2002). Furthermore, in the femoral artery of the rat the lack of MEGJs is correlated with the absence EDHF-mediated hyperpolarisation (Sandow *et al.*, 2002). In the lateral saphenous artery of the juvenile rat, MEGJs are prevalent and EDHF-mediated hyperpolarisation and relaxation present (Sandow *et al.*, 2003a). This is in contrast to the saphenous artery of the adult, where MEGJs were rare and EDHF absent (Sandow *et al.*, 2003a). The relationship between EDHF and MEGJs is somewhat more complicated in disease states, such as in hypertension. In a comparative study of the caudal artery of the hypertensive SHR and normotensive WKY rat, EDHF activity was maintained, in spite of an increase in the number of smooth muscle cell layers in the vessels from the hypertensive rat. This maintenance was found to be due to a concomitant increase in the incidence of MEGJs in the caudal artery of the SHR rat (Sandow *et al.*, 2003b).

These studies demonstrate that there is a consistent positive correlation between MEGJs and EDHF activity, both of which show a heterogeneous distribution within and between vascular beds, during development and in disease. Thus, these studies demonstrate that heterocellular coupling can account for EDHF activity. Further studies will enable the identification of potential new therapeutic targets for the regional control of vasodilator function, vascular tone and cardiovascular disease.

Sandow, S.L. & Hill, C.E. (2000) *Circulation Research,* 86, 341-346.

Sandow, S.L., Tare, M., Coleman, H.A., Hill, C.E. & Parkington, H.C. (2002) *Circulation Research,* 90, 1108-1113.

Sandow, S.L., Bramich, NJ, Bandi, HP, Rummery, N & Hill, C.E. (2003a) *Arteriosclerosis, Thrombosis, and Vascular Biology*, 23, 822-828.

Sandow S.L., Goto, K, Rummery, N & Hill, C.E. (2003b). FEPS Abstract. 3, 100.

Shimokawa, H., Yasutake, H., Fujii, K., Owada, M.K., Nakaike, R., Fukumoto, Y., Takayanagi, T., Nagao, T., Egashira, K., Fujishima, M. & Takeshita, A. (1996) *Journal of Cardiovascular Pharmacology,* 28, 703-711.

Endothelial potassium channels in the regulation of vascular tone in health and in disease

H.A. Coleman, M. Tare and H.C. Parkington, Department of Physiology, Monash University, Vic 3800, Australia.

Ionic mechanisms underlying EDHF. The elusive nature of endothelium-derived hyperpolarising factor (EDHF) has hampered detailed study of the underlying ionic mechanisms. By cutting arterioles into electrically short lengths it is possible to record membrane currents using single electrode voltage-clamp when the smooth muscle and endothelial cells remain in their normal functional relationship. Membrane potential can also be recorded simultaneously with contractile activity in these preparations. With this approach it is thus possible to study endothelial-dependent ionic mechanisms irrespective of the processes involved and to relate the currents to contractile activity.

In the presence of nitric oxide and prostaglandin synthesis inhibitors, acetylcholine (ACh) evoked hyperpolarisation and relaxation of guinea-pig submucosal arterioles which were abolished by the K^+ channel blockers charybdotoxin (ChTx) plus apamin. Under voltage-clamp, ACh evoked an outward current. ChTx reduced the amplitude, while apamin plus ChTx abolished the outward current. Subtraction of the currents revealed the ChTx- and apamin-sensitive currents and their separate current-voltage relationships. Both currents reversed near the expected K^+ equilibrium potential, were weakly outwardly rectifying, and displayed little, if any, time or voltage-dependent gating. The components have the biophysical and pharmacological characteristics of the intermediate- and small-conductance calcium-activated K^+ channels, IK_{Ca} and SK_{Ca}, respectively (Coleman *et al.*, 2001).

Myoendothelial electrical coupling. Electrotonic spread between endothelial and smooth muscle cells is an important consideration for EDHF. Smooth muscle specific responses recorded from dye-labelled endothelial cells were indistinguishable from those recorded from dye-labelled smooth muscle cells. In contrast, in rat femoral artery, in which the smooth muscle and endothelial layers are not coupled electrically, ACh evoked hyperpolarisation only in endothelial cells. This supports the idea that EDHF hyperpolarisation results from electrotonic spread from the endothelium to the smooth muscle (Coleman *et al.*, 2001; Sandow *et al.*, 2002).

EDHF *in vivo*. The functional significance of EDHF *in vivo* was addressed by the local infusion of ACh into the rat mesenteric vascular bed. With nitric oxide and prostaglandin synthesis blocked, ACh evoked increases in blood flow that were blocked with the local infusion of ChTx plus apamin. These results indicate that EDHF contributes to endothelium-dependent vasorelaxation *in vivo* (Parkington *et al.*, 2002).

EDHF in diabetes. Vasodilator dysfunction is a well established hallmark of diabetes. In arteries from diabetic rats and women, EDHF is diminished. This is not only associated with reduced EDHF hyperpolarisation in vascular smooth muscle, but is also associated with a reduced hyperpolarisation in the endothelial cells (Wigg *et al.*, 2001).

In conclusion, the most economical explanation for EDHF is that it arises from activation of IK_{Ca} and SK_{Ca} channels in endothelial cells. The resulting endothelial hyperpolarisation spreads via myoendothelial junctions to result in the EDHF-attributed hyperpolarisation in vascular smooth muscle cells. These processes contribute to endothelium-dependent vasodilation *in vivo* and their dysfunction contributes to the impairment of vascular regulation that occurs in diabetes.

Coleman, H.A., Tare, M. & Parkington, H.C. (2001). *Journal of Physiology*, 531(2), 359-373.

Parkington, H.C., Chow, J.-A.M., Evans, R.G., Coleman, H.A. & Tare, M. (2002). *Journal of Physiology*, 542, 929-937.

Sandow, S.L., Tare, M., Coleman, H.A., Hill, C.E. & Parkington, H.C. (2002). *Circulation Research*, 90, 1108-1113.

Wigg, S.J., Tare, M., Tonta, M.A., O'Brien, R.C., Meredith, I.T. & Parkington, H.C. (2001). *American Journal of Physiology*, 281, H232-H240.

Changes in endothelium-derived hyperpolarising factor in ageing and hypertension: response to chronic treatment with renin-angiotensin system blockers

K. Goto, K. Fujii, Y. Kansui and M. Iida, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka, 812-8582, Japan. (Introduced by Prof. Caryl Hill)

Endothelial cells play an important role in the regulation of vascular tone through the release of relaxing factors such as nitric oxide, prostacyclin, and endothelium-derived hyperpolarising factor (EDHF). EDHF appears to be a dominant vasodilator in resistance arteries although its identity is still elusive. Several clinical and experimental studies have shown that endothelial function is impaired in ageing and hypertension, which may be associated with an increase in cardiovascular disease. In addition, several clinical studies have shown that blocking the renin-angiotensin system (RAS) improves endothelial function not only in hypertensive patients but also in normotensive patients with other cardiovascular diseases, such as chronic heart failure and/or myocardial infarction. The aim of the present study was to test whether or not EDHF-mediated hyperpolarisation and relaxation change in ageing and hypertension, and if so, whether or not chronic treatment with RAS blockers (an angiotensin-converting enzyme inhibitor enalapril and an angiotensin II receptor antagonist candesartan) improves such change. EDHF-mediated hyperpolarisation and relaxation were examined in mesenteric arteries obtained from 3-, 6-, 12-, and 24-month-old normotensive Wistar-Kyoto rats (WKY) and 12-month-old spontaneously hypertensive rats (SHR). Furthermore, both strains were treated for three months with either RAS blockers or a conventional therapy with hydralazine and hydrochlorothiazide from 9- to 12-month-old. The rats used were anaesthetised with ether and killed by decapitation. In arteries of WKY, EDHF-mediated hyperpolarisation and relaxation were impaired at the age of 12- and 24-months compared with 3- and 6-month-old rats, with the response tending to be further impaired in 24-month-old rats. Three months of treatment with RAS blockers but not with a conventional therapy with hydralazine and hydrochlorothiazide improved the age-related impairment of EDHF-mediated responses, despite a similar reduction in blood pressure in both treatments. In arteries of SHR, EDHF-mediated hyperpolarisation and relaxation were impaired at the age of 12-months compared with age-matched, 12-month-old WKY. In SHR, all antihypertensive treatments improved the impairment of EDHF-mediated responses; however, the improvement achieved by RAS blockers was greater than that with a conventional therapy with hydralazine and hydrochlorothiazide. These findings suggest that: (1) EDHF-mediated hyperpolarisation and relaxation decline with ageing and hypertension in rat mesenteric arteries; (2) chronic treatment with RAS blockers improves the age-related impairment of EDHF-mediated responses presumably through the blockade of RAS but not lowering the blood pressure alone; (3) antihypertensive treatment restores the impaired EDHF-mediated responses in hypertension; and (4) RAS blockers may be more efficacious in improving the endothelial dysfunction associated with hypertension.

Potassium channels in the cerebral circulation in health and vascular disease

C.G. Sobey, Department of Pharmacology, The University of Melbourne, Parkville, Victoria 3010, Australia. (Introduced by C.E. Hill)

Vascular K^+ channel function. Potassium ion (K^+) channel activity is a major regulator of vascular smooth muscle cell membrane potential, and is therefore an important determinant of vascular tone. Several diverse endogenous vasodilator stimuli act at least in part via activation of vascular K^+ channels. The function of several types of vascular K^+ channels is altered during major cardiovascular diseases, such as hypertension, atherosclerosis, diabetes and subarachnoid haemorrhage (SAH). Vasoconstriction and compromised ability to dilate are likely consequences of defective K^+ channel function in blood vessels during these disease states. Increased K^+ channel function may help to compensate for excessive vascular tone. In recent years our laboratory has investigated the functional importance of K^+ channels in the cerebral circulation in physiology and during SAH and chronic hypertension.

Reactive Oxygen Species (ROS) as openers of K^+ channels. ROS are powerful cerebral vasodilators and mediators of responses to bradykinin and arachidonate. Both agents produce endothelium-dependent dilatation of cerebral arterioles that is indomethacin- and catalase-sensitive, indicating that cyclooxygenase-derived ROS mediate these responses. Dilatation of cerebral arterioles by bradykinin, arachidonate or exogenous hydrogen peroxide (H_2O_2) can be blocked using tetraethylammonium (TEA) or iberiotoxin, suggesting a key role for activation of large conductance calcium-activated K^+ (BK_{Ca}) channels.

Extracellular K^+. Raising extracellular K^+ concentration from approx. 3-5 mM to ≤15 mM increases outward K^+ current through inwardly rectifying K^+ (K_{IR}) channels, causing vascular smooth muscle hyperpolarisation and relaxation. K^+ is a particularly powerful dilator in the cerebral circulation, and its effect is selectively inhibited by barium ion (≤ 50 μM) indicating an involvement of K_{IR} channels. Our recent data indicate that K^+ is a more potent vasodilator in cerebral arteries of females than males.

K^+ channel function after SAH. After SAH, bleeding and clot formation occur around the ventral surface of the brain, including major arteries, often resulting in death or severe disability. Delayed spasm and impaired dilatation of the affected arteries are critical complications of SAH. These cerebral arteries are more depolarised than control vessels, possibly due to decreased activity of K^+ channels in vascular muscle. Vasodilator drugs which produce hyperpolarisation, such as K^+ channel openers, appear to be effective for dilating cerebral arteries after experimental SAH.

NADPH-oxidase, ROS and Hypertension. NADPH, a substrate for NADPH-oxidase, stimulates superoxide production in basilar arteries which is blocked by diphenyleneiodonium (DPI, a NADPH-oxidase inhibitor), and this production is >2-fold higher in SHR versus WKY rats. Cerebral artery mRNA expression of the NADPH-oxidase subunit, Nox4, is 4-fold higher in SHR. Application of NADPH to the basilar artery *in vivo* causes greater dilatation in SHR than WKY. DPI or inhibitors of superoxide dismutase (diethyldithiocarbamate, DETCA), H_2O_2 or BK_{Ca} channels attenuate NADPH-stimulated vasodilatation. Interestingly, bilateral carotid artery occlusion to increase flow in the basilar artery induces nitric oxide-independent vasodilatation that is inhibited by DPI. Thus, a novel mechanism for ROS-mediated vasodilatation appears to exist in the cerebral circulation in response to NADPH or increased flow, whereby NADPH-oxidase-derived superoxide is reduced by SOD to form H_2O_2. H_2O_2 then opens BK_{Ca} channels, leading to vasodilatation. Furthermore, cerebral NADPH-oxidase activity is augmented during chronic hypertension.