AuPS/ASB Meeting - Canberra 2005

Free communications 12: Systems physiology

Friday 30 September 2005

Chair: Rick Lang

Novel nifedipine-insensitive high voltage activated calcium channels play a role in vascular tone of cerebral arteries

M.F. Navarro-Gonzalez and C.E. Hill, Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia.

Calcium channels are common therapeutic targets for the treatment of cardiovascular disorders such as hypertension (Ishikawa *et al.*, 1997), however they have not always been as successful as might be expected against vasospasm and stroke. Recently a novel high voltage activated, nifedipine-insensitive, mibefradil-sensitive calcium channel was described in small mesenteric arteries of guinea pigs and rats (Morita *et al.*, 1999, 2002), and called the "M-type" voltage dependent calcium channel (mVDCC). This channel has also been found in rabbit mesenteric arteries, where it is suggested to play a role in diameter regulation (Itonaga *et al.*, 2002). The aim of the present study was to determine if cerebral arteries possess similar nifedipine-insensitive VDCCs which could be used as targets for cerebrovascular disorders.

Juvenile (14-17 day old) male Wistar rats were anaesthetized with ether and decapitated. The basilar artery was removed from the brain and superfused with physiological Krebs solution at 33-37°C. Diameter was monitored as a measure of vascular tone using an edge-tracking computer program. Membrane potential was measured with sharp intracellular microelectrodes (100-180 MΩ), and current pulses (1-2 min) were applied to short segments of artery (less than 800µm) using discontinuous current clamp mode (Axoclamp 2B). Change in intracellular calcium concentration ($[Ca]_i$) was measured with the ratiometric calcium indicator Fura-2 AM and a photometry system. After 30 minutes the arteries developed spontaneous rhythmical oscillations in diameter (vasomotion) and membrane potential with the most negative potential around -45mV. Application of the L-type VDCC blocker, nifedipine, abolished vasomotion but did not alter tone, membrane potential or $[Ca]_i$ in basilar arteries, while inhibition of the IP_3 pathway with U73122 also abolished vasomotion but caused hyperpolarization, relaxation and a decrease in $[Ca]_i$. Small hyperpolarizing current steps which took the membrane potential to -50mV caused immediate abolition of vasomotion and relaxation. Relaxation occurred in the presence or absence of nifedipine. Application of the T- and M-type VDCC blocker, mibefradil, hyperpolarized and relaxed the artery, decreasing $[Ca]_i$, while the T-type VDCC blocker, nickel chloride, only relaxed the artery at a high non-specific concentration (1mM). After the artery was hyperpolarized and relaxed with U73122, application of 40mM KCl caused depolarization and constriction in the presence of nifedipine. A similar result was obtained when 2-APB, an IP_3 inhibitor was present together with nifedipine, suggesting that the effect of voltage was not on calcium release from intracellular stores. Taken together the results suggest that nifedipine-insensitive, mibefradil-sensitive VDCCs play a role in vascular tone in the rat basilar artery. These channels are activated at depolarized potentials and rapidly closed by small hyperpolarizations. They are thus unlikely to be T-type VDCCs, which are activated at more negative potentials and rapidly inactivated during prolonged depolarization. We suggest that these nifedipine-insensitive high voltage-activated calcium channels may provide a novel therapeutic target for cerebrovascular disorders.

Ishikawa, K., Nakai, S., Takenaka, T., Kanamasa, K., Hama, J., Ogawa, I., Yamamoto, T., Oyaizu, M., Kimura, A., Yamamoto, K., Yabushita, H. & Katori, R. (1997) *Circulation*, **95**, 2368-2373.

Itonaga, Y., Nakajima, T., Morita, H., Hanano, T., Miyauchi, Y., Ito, Y. & Inoue, R. (2002) *Life Sciences*, **72**, 487-500.

Morita, H., Cousins, H., Onoue, H., Ito, Y. & Inoue, R. (1999) *Circulation Research*, **85**, 596-605.

Morita, H., Shi, J., Ito, Y. & Inoue, R. (2002) *British Journal of Pharmacology*, **137**, 467-476.

Accentuation during diabetes of differential connexin expression between the preglomerular and postglomerular renal vasculature

J.H. Zhang and C.E. Hill, Division of Neuroscience, JCSMR, ANU, Acton, ACT 2602, Australia.

Gap junctions may play an important role in regulating renal blood flow and glomerular responses. Our previous studies have demonstrated extensive expression of connexins (Cxs) 37, 40 and 43 in endothelial cells and Cx37 in smooth muscle cells of the preglomerular renal vasculature and of Cxs37 and 40 in the renin-secreting cells and intraglomerular mesangial cells. In contrast, there was limited cell coupling in the efferent arterioles with only Cx43 found in the endothelium (Zhang & Hill, 2004). Since elevated glucose has been reported to down-regulate Cx43 in vascular cells *in vitro* (Kuroki *et al.*, 1998; Sato *et al.*, 2002), our aim was to determine the impact of diabetes on Cx expression in the renal vasculature. Diabetes was induced with sequential daily doses of streptozotocin in citrate buffer (120/80 mg/kg, pH 4.4, intraperitoneally) in male C57BL/6 mice (8-10 weeks) while vehicle injected mice were used as controls. Diabetes was defined as a nonfasting blood glucose level ≥ 18 mmol/L on two consecutive days. Mice were deeply anaesthetized (rompun/ketamine 5/25mg/kg body wt. i.p.), the kidneys removed, fixed in ice-cold acetone and 30 μm coronal cryosections cut. Cx distribution was determined at 2, 4, 6, 8 and 10 weeks after the onset of diabetes, using immunohistochemistry and Cx subtype-specific and celltype-specific antibodies. Quantification of Cx changes associated with diabetes was made using the software program Analytic Imaging Station 3. At 2 weeks of diabetes, Cx43 expression in the endothelium of the efferent arterioles was reduced and in many glomeruli was absent by 8 weeks. By 4 weeks, the glomeruli had increased in size and the expression of Cx37 in mesangial cells within the glomerulus had expanded from the vascular pole, while there was no change in Cx37 in the preglomerular vasculature. Cx40 expression in the glomerulus was also increased but not when considered in relation to the enlarged size of the glomerulus. Cx40 was now found in the smooth muscle cells of the afferent arterioles. These changes in Cx expression were maximal by 8 weeks. At 10 weeks of diabetes, Cx43 was detected in the renin secreting cells and in the adjacent smooth muscle cells of the afferent arterioles. We conclude that, during diabetes, cellular coupling within the preglomerular vasculature and intraglomerular mesangial cells is increased while the restricted coupling on the postglomerular side is further reduced. We propose that these changes may accentuate the independence of responses in afferent and efferent arterioles and contribute to the hyperfiltration and pathophysiological damage seen in the diabetic kidney.

Kuroki, T., Inoguchi, T., Umeda, F., Ueda, F & Nawata, H. (1998) *Diabetes* **47**, 931-936.
Sato, T., Haimovici, R., Kao, R., Li, A.F. & Roy, S. (2002) *Diabetes* **51**, 1565-1571.
Zhang, J.H. & Hill, C.E. (2004) *Proceedings of the Australian Physiological Society*, **35**, 87P.

High-amplitude oscillations in human skin blood flow are distinct from known cardiac or respiratory influences

C.D. Haley, C.J. Gordon, N.A. S. Taylor and A.B. Jenkins , Department of Biomedical Science, University of Wollongong, Wollongong, NSW 2522, Australia.

We have recently described the presence of high-amplitude skin blood flow ($\dot{Q}_{sk}$) oscillations in humans (Haley *et al*., 2004a) and animals (Haley *et al*., 2004b). These oscillations had a characteristic frequency of ~0.4 Hz, spanning ~700-800 ms, and were comprised of high-amplitude peaks that are up to 7-fold greater than basal $\dot{Q}_{sk}$. We hypothesised that the high-amplitude oscillations may be related to previously identified lower-amplitude (±30 %) oscillations in $\dot{Q}_{sk}$ caused by respiration (0.15-0.4 Hz) and cardiac frequency (0.4-1.6 Hz) (Stefanovska *et al*., 1999). Hence, we sought to compare the spectra of respiratory and cardiac activities, with the $\dot{Q}_{sk}$ time-frequency spectrum.

Forearm $\dot{Q}_{sk}$ (ventral aspect), cardiac frequency and respiration were measured simultaneously (20 Hz) from eight males (27.9 yr (SD 6.4), 181.1 cm (SD 4.8), 75.8 kg (SD 7.5), during semi-recumbent rest at 25°C (50% R.H.), on two separate days, for a 5-6 min period. Skin blood flow, was estimated using laser-Doppler flowmetry (TSI Laserflo BPM2, Vasamedics Inc., U.S.A.), cardiac frequency was collected using a three-lead electrocardiogram (ECG: model 100, Humtec, Australia) and respiration was measured from rib cage movement (mercury-in-silastic strain gauge: Hokansen EC-4SB, U.S.A.). Respiratory, ECG and $\dot{Q}_{sk}$ data were analysed using a wavelet transform in the frequency domain of 0.05-2 Hz; the dominant frequency band for each variable was calculated as the central portion accounting for 95% of the total integrated power of the time-averaged frequency spectrum. Data are means ± standard errors.

The dominant frequency band for the $\dot{Q}_{sk}$ spectrum (0.72 ±0.03) was significantly different (P<0.05, paired t-test) from both the respiratory (0.22 ±0.02) and ECG spectra (1.31 ±0.01). Variations between studies (n=16) in the $\dot{Q}_{sk}$ dominant frequencies were not significantly correlated with between study variations in either cardiac (r=0.02, P =0.94) or respiration (r=-0.15, P=0.60) dominant frequencies. These results indicate that the high-amplitude $\dot{Q}_{sk}$ oscillations are not directly related to either cardiac frequency or respiratory function. Instead, we propose that these oscillations are related to local factors, such as changes in transmural pressure, or the release of substances that alter vascular endothelial and smooth muscle function.

Haley, C.D., Zeyl, A., Taylor, N.A.S. & Jenkins, A.B. (2004a) *Journal Thermal Biology*, 29, 717-723.
Haley, C.D., Gordon, C.J., Taylor, N.A.S. & Jenkins, A.B. (2004b) *Journal Thermal Biology*. 29, 779-783.
Stefanovska, A., Bracic, M & Kvernmo, H.D. (1999) *IEEE Transactions on Biomedical Engineering*, 46(10), 1230-1239.

Activation of at least three classes of ion channels by β-adrenoceptor activation in pregnant uterine smooth muscle

H.C. Parkington, M.A. Tonta, S. Simon, S.A. Cohen, A. Satragno, R.J. Lang and H.A. Coleman, Department of Physiology, Monash University, Vic 3800, Australia.

Preterm labour complicates 5-10% of births, has significant repercussions for neonatal morbidity and mortality and may have consequences for lifelong health. Agents that stimulate β-adrenoceptors are commonly used to suppress preterm uterine contractions, yet despite considerable effort, a complete understanding of the mechanisms involved is lacking. We have previously shown that activation of β-adrenoceptors in sheep myometrium markedly reduces the sensitivity of the contractile apparatus to Ca^{2+} and induces large hyperpolarization that is inhibited by blockade of ATP-sensitive K^+ (K_{ATP}) channels (Parkington *et al*., 2000). Stimulation of β-adrenoceptors shifts the activation curve for large-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channels to the left in human myometrium (Zhou *et al*., 2000).

In the present study we probed the effects of β-adrenoceptor activation in late pregnant sheep myometrium using a variety of approaches: (1) simultaneous recording of membrane potential and tension in myometrial strips; (2) patch clamp recordings of single channel and whole cell currents in freshly isolated cells from these same ewes; and (3) simultaneous recording of extracellular electrical (EMG) and contractile activity in the uterus of conscious ewes at days 130-140 of pregnancy (term ~145 days). Under general halothane anaesthesia and using full sterile techniques, EMG electrodes and transducers were attached to the uterus and catheters implanted into a branch of the uterine artery and the jugular vein for local and general drug infusion, respectively. A fetal jugular catheter was implanted to monitor fetal well being. Isolated tissues were obtained during surgery and again at *post mortem*. Labour was induced preterm in 5 ewes by infusion of RU486 (0.5 mg/kg) (Hirst *et al*., 2005). Salbutamol was used to stimulate β-adrenoceptors.

The hyperpolarization (15 mV in tissues from 20 ewes) evoked in cells in myometrial strips by salbutamol (10-300 nM) was blocked by glibenclamide (1 μM, n=16) and PNU-37883A (10 μM, n=4) but not by iberiotoxin (60 nM, n=3) or charybdotoxin (60 nM, n=6), indicating activation of K_{ATP} but not BK_{Ca} channels. However, salbutamol induced a prominent activation of BK_{Ca} channels in isolated cells and caused a leftward shift of the activation curve that was similar to raising the intracellular Ca^{2+} concentration (n=12). Blockade of BK_{Ca} channels with iberiotoxin revealed that salbutamol also activated two small channels, a K_{ATP} channel of 62pS and a channel (conductance 14 pS) that reversed near −20 mV. In strip preparations continuously superfused with glibenclamide-containing solution, the amplitude of the action potential was reduced by salbutamol, and this was blocked by iberiotoxin. In addition, in the presence of PNU-37883A, salbutamol induced a small depolarization.

Infusion of salbutamol (100 μg/kg/h) into conscious ewes, 7 days after surgery, caused immediate cessation of the bursts of EMG activity and associated contraction that occurs 3-4 times per hour. Following 30 min infusion of glibenclamide (1mg/kg/h), salbutamol failed to suppress uterine activity before labour (n=7), during normal spontaneous labour (n=3), and following induction of labour preterm (n=5).

These results demonstrate a significant activation of K_{ATP} by salbutamol in pregnant sheep myometrium at the single channel and tissue levels and in the intact ewe. BK_{Ca} channels are also activated, and their main effect is to reduce the amplitude of the action potential and not to cause membrane hyperpolarization. An intriguing and unexpected action of salbutamol was the activation of an inward conductance, but its role in the excessive "rebound" uterine activity observed following salbutamol withdrawal *in vivo* awaits further investigation.

Hirst, J.J., Parkington, H.C., Young, I.R., Palliser, H.K., Peri, K.G. & Olson, D. (2005) *American Journal of Obstetrics and Gynecology* (in press)

Parkington, H.C., Tonta, M.A. & Coleman, H.A. (2000) *Proceeding of the Australian Physiological and Pharmacological Society* **31**, 15P.

Zhou, X.B., Wang, G.X., Ruth, P., Huneke, B. & Korth, M. (2000) *American Journal of Physiology* **279**, C1751-1759.

Expression of a constitutively active K^+ channel prevents cell division in the mouse preimplantation embryo

M.L. Day[1], C.G. Bailey[2], J.E. Rasko[2], M.H. Johnson[3] and D.I. Cook[1], [1]Physiology, School of Medical Sciences, University of Sydney, NSW 2006, Australia, [2]Gene Therapy, Centenary Institute of Cancer Medicine & Cell Biology, University of Sydney, NSW 2042, Australia and [3]Department of Anatomy, Downing St., University of Cambridge, CB2 3DY, UK.

The activity of a large-conductance, voltage-gated K^+ channel changes during the cell cycle in all stages of mouse preimplantation development. This channel is active during M and G1 phases and inactive during S and G2. In parallel with the oscillations in K^+ channel activity are changes in the cell membrane potential, being hyperpolarized when the channel is active. The channel appears to be regulated by a cytoplasmic cell cycle that can function independent of the activation of the Cdk1/Cyclin B complex, but does also interact with the nuclear cell cycle. The objective of this study was to determine whether the cycling of K^+ channel activity in the mouse early embryo is required for progression of the cell cycle. In these studies we used an adenoviral construct containing a constitutively active mutant of the K^+ channel IRK1 (D172N-IRK1) and GFP under separate promoters. A control adenoviral construct that only contained GFP was used to determine non-specific effects of adenovirus transduction. Quackenbush strain mice were superovulated by intraperitoneal injections of pregnant mares' serum gonadotrophin and human chorionic gonadotrophin (hCG) 48 hours apart. Mice were killed by cervical dislocation approximately 48 hours after hCG injection and 2-cell embryos isolated. Embryos were then transduced with the adenoviruses by incubation in medium M16 containing 1×10^5 pfu/ml adenovirus. Successful transduction of embryos was determined after 16 hours by the expression of GFP. Whole-cell patch-clamping confirmed the expression of an inwardly-rectifying K^+ current in the GFP positive 4-cell embryos. Expression of D172N-IRK1 caused the membrane potential to be hyperpolarized (-59.1 mV) compared with the membrane potential in non-transduced embryos (-34.7 mV). Development of embryos to the 8-cell stage was reduced from 76.9% in non-transduced embryos to 16.3% in embryos expressing D172N-IRK. These results suggest that cyclic changes in K^+ channel activity are important for cell cycle progression in the early embryo. Whether the inhibitory affect is due to hyperpolarization of the membrane potential or loss of cytosolic K^+ requires further study.