The High Blood Pressure Research Council of Australia would like to acknowledge the support from Servier Laboratories enabling this publication.
INSULIN REGULATED AMINOPEPTIDASE/AT1 RECEPTOR DEFICIENCY IS BOTH CARDIO- AND VASO-PROTECTIVE IN ANGIOTENSIN II-INFUSED MICE

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Chronic treatment with the hexapeptide 3–8 fragment of Angiotensin (Ang) II, known as Ang IV, mediates vasoprotective effects in the ApoE KO mouse model of atherosclerosis and we propose that these effects are mediated by the binding of Ang IV to the AT4 receptor, now identified as insulin-regulated aminopeptidase (IRAP), inhibiting the catalytic activity of this enzyme. Therefore the objective of this study was to investigate whether IRAP deficiency confers a protective phenotype under condition of cardiovascular stress. Using the Ang II-infusion model of hypertension, male IRAP-deficient (IRAP–/–) mice and their WT littermate controls (IRAP+/+) were treated with either Ang II (800 ng/kg/day) or saline subcutaneously via osmotic mini-pumps for 4 weeks. Ang II-treated WT and IRAP−/− mice had a significant increase in systolic blood pressure (141 ± 4 mm Hg and 149 ± 8 mm Hg, respectively; n = 8–11, P < 0.01) compared to vehicle treated mice, with a concomitant increase in HW-BW ratio. Ang II-infused WT mice had impaired endothelium-dependent vasorelaxation (Rmax: 44.2% ± 6.0%; n = 7) compared to saline-treated WT mice (Rmax: 69.5 ± 7.3%; n = 6, P < 0.01). Interestingly Ang II-infused IRAP−/− mice showed no evidence of endothelial dysfunction (Rmax: 63.9% ± 5.1%; n = 6).

Ang II-induced endothelial dysfunction was correlated with a reduction in eNOS immuno-staining in Ang II-infused WT mice that was prevented in IRAP−/− mice. Ang II-infusion evoked cardiac fibrosis in WT mice with an increase in collagen deposition (3.41 ± 0.33%; n = 5; P < 0.001) compared to saline treated WT mice (0.86 ± 0.10%; n = 5). Excitingly, IRAP−/− mice were protected against development of increased cardiac interstitial fibrosis (0.85 ± 0.04%; n = 5; P < 0.001) when treated with Ang II for 4 weeks. In conclusion this study has shown that IRAP−/− mice possess both vas- and cardio-protective phenotype under condition of cardiovascular stress and thus highlights the importance of targeting the IRAP/AT4R in cardiovascular disease.

EXERCISE HYPERTENSION IS RELATED TO AORTIC RESERVOIR FUNCTION: A FIRST IN-HUMAN EXERCISE CENTRAL HAEMODYNAMIC STUDY

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Exercise hypertension is prognostically important, but little is known on central haemodynamic factors associated with this response. We hypothesised that increased exercise central blood pressure (BP) would be related to increased forward wave travel and proximal aortic reservoir function. This study aimed to determine this using wave intensity analysis (WIA) for the first time during exercise. Following routine diagnostic coronary angiography, simultaneous pressure and Doppler flow velocity waveforms were recorded in the ascending aorta via sensor-tipped intra-arterial wires in 10 participants (mean age 54 ± 10 years, 70 % male) with normal left-ventricular function and who were free of coronary artery disease. Measures were recorded at baseline and during light-moderate intensity cycle exercise at 60% of age-predicted-maximum heart rate (HR). Aortic reservoir pressure was calculated by subtraction from the central pressure waveform. Using WIA we identified dominant wave types throughout the cardiac cycle (forward and backward, compression and decompression waves). From rest to exercise, HR and maximal central BP increased significantly (+28 % and +15 %, P < 0.001 for both respectively). The strongest correlate of the change in maximal BP was the change in peak aortic reservoir pressure (r = 0.755, P = 0.012). During exercise, there were significant increases in forward compressive waves during early systole (baseline 27.02 ± 10.55 mm Hg vs. exercise 41.01 ± 10.52 ≈ 21.09 mm Hg: Wm−2 z−2; + 62 %, P = 0.014, corresponding to peak ejection) and forward decompression waves in late systole (baseline 10.96 ± 4.60 ≈ 6.40 mm Hg vs. exercise 20.88 ± 5.79 ≈ 10.09 mm Hg: Wm−2 z−2; + 121 % change P < 0.001 (corresponding to myocardial deceleration pre-aortic valve closure), but no change in backward travelling waves (P = 0.05 for all). We conclude that increased central BP with exercise may be due to impaired aortic reservoir function and major increases in aortic forward travelling waves that occur in early systole. These findings have relevance to understanding the pathophysiology of exercise hypertension.

PREDICTING PREGNANCY OUTCOMES: SMALL OR EARLY?

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Being born small or early clearly has important implications for lifelong health. Placental differentiation and function are essential for optimal fetal growth and development and when impaired predispose the pregnancy to a variety of complications including intrauterine growth restriction (IUGR) and preterm birth (PTB). The challenge is to identify women in early pregnancy who are at risk so that interventions can be made early enough to improve placentation. We recruited 3224 nulliparous women before 15 weeks gestation in Adelaide and Auckland. Both parents were Caucasian in 2123 pregnancies. Blood samples were collected and DNA extracted from mother-father-baby trios. Genotyping of single nucleotide polymorphisms (SNPs) in genes that affect placental development and maternal adaptation to pregnancy and logistic regression analyses have identified a number of SNPs in VEGF family, renin angiotensin (RAS) family and IGF family genes that interact with the environment to predict risk for IUGR and PTB. Furthermore, fetal sex affects the association of RAS genes in pregnancy outcome, with the female fetus being at risk in particular. Maternal socioeconomic status, diet and BMI interact with SNPs to influence risk. This research has identified genetic and lifestyle factors that may be amenable to future intervention and simple modification.

DO CORTICOSTEROIDS HAVE DIFFERENT EFFECTS IN PRETERM OR SMALL BABIES?

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Fetal glucocorticoid exposure commonly occurs in women threatening to deliver their baby prematurely. This treatment has proved efficacious in preventing much of the morbidity and mortality associated with life threatening respiratory problems faced by premature delivery. However, it is now recognised that prenatal glucocorticoid exposure can have deleterious effects on the development of other organs (such as the kidney and the heart)
which may in the longer term contribute to adult onset disease, including hypertension. Recent data have suggested that neonatal outcomes following prenatal glucocorticoid exposure may in part be dependent upon fetal sex with the placenta playing a role in modifying the amount of corticosteroid that reaches the fetal circulation. Also of recent concern are data suggesting that small for gestational age (SGA) babies may respond quite differently to glucocorticoid exposure compared to an appropriately grown baby. Given that many babies being born prematurely are also growth restricted it is particularly pertinent to consider the impact of glucocorticoid exposure in SGA babies. Our own emerging data in mouse models suggest that maternal glucocorticoid treatment has differential effects on developing fetal organs such as the kidney, heart and skin depending upon pup size. This suggests future treatment of a mother with prenatal glucocorticoids should take into account the fetal size.

**PATHWAYS FROM BEING SMALL OR PRETERM TO A VULNERABLE HEART**

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Left ventricular hypertrophy is an indicator of poor cardiovascular outcome. It is possible that slow growth in fetal life upregulates cardiac signaling pathways involved in left ventricular hypertrophy and thus puts the individual at risk of further cardiovascular disease in adult life. We have shown that the IUGR fetus has higher cardiac IGF-2 and IGF-2R gene expression. This may be a response to the chronic hypoxia that these fetuses are exposed to. If this is the case, it is not clear why this change in cardiac gene expression is maintained into postnatal life fetal to birth weight lamb at 21d of age, at a time when oxygen and nutrient supply are normal. In vitro rat experiments have shown that IGF-2R activation can induce cardiomyocyte hypertrophy via a G protein coupled receptor (Gaq)-dependent manner. Does the increase in IGF-2R gene expression represent increased clearance of IGF-2 or does IGF-2R activate pathological hypertrophy signaling pathways in the heart of the intrauterine growth restricted (IUGR) fetus and the low birth weight lamb? Cardiomyocytes from sheep fetuses at 126d gestation were isolated and cultured in the presence of Leu27IGF-2, to selectively activate the IGF-2 signalling pathway, and inhibitors of protein kinase C (PKC) were added to this pathway. Leu27IGF-2 increased the area of cultured birefringent, but not mononucleated, cardiomyocytes. Inhibition of PKC with Gö6976 did not prevent the Leu27IGF-2 induced increase in the cell area of birefringent cardiomyocytes; however, inhibition of CaMKII with KN-93 blocked the effect of Leu27IGF-2. These data suggest that activation of IGF-2R increases cardiomyocyte area via CaMKII in vitro. To confirm these effects in the normally grown fetus in late gestation, we inserted a catheter into the left circumflex coronary artery to infuse Leu27IGF-2 selectively to the left ventricle. Infusion of Leu27IGF-2 did not change fetal weight or heart weight. Importantly, Leu27IGF-2 did not increase blood pressure, the major contributor to hypertrophy of cardiomyocytes. There was no effect cardiomyocyte proliferation or binucleation, but there was an increase in the area of cardiomyocytes. These data show that both in vitro and in vivo, activation of cardiac IGF-2R signalling pathway in the fetus results in hypertrophic growth of cardiomyocytes. The specific proteins mediating these effects are unclear and require elucidation.

**PRETERM NEONATAL CARDIOVASCULAR INSTABILITY: UNDERSTANDING THE FETUS WHEN EVALUATING THE NEWBORN**

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Poor perfusion of the kidneys and gut, and associated functional impairment, are major problems in the first days of life in very premature infants. These complications can be associated with substantial mortality and further problems such as reduced kidney growth and other growth problems in later life. There is very little information, and consequently considerable debate, about how or even whether to improve perfusion of the vital organs of this most vulnerable group of babies. Indeed, significant variability exists in treatment options, and many therapies lack robust validation. Current treatments simply do not consistently improve babies' perfusion generally or kidney and gut perfusion and function in particular. In many infants, low blood flow is not mediated by low blood pressure. Rather hypoperfusion may be secondary to actively mediated vasoconstriction, which may have been initiated in utero as part of the fetal responses to hypoxia, and which is still active after birth as part of post-hypoxic recovery. Thus while the fetus has transitioned to newborn life, the responses must be considered as part of a continuum of responses from fetus to newborn. Most important are the post-hypoxic responses of the fetus, the mechanisms mediating hypoperfusion, and the role vasoconstriction plays in supporting perfusion of the kidneys and other vital organs in premature babies.

**THE ROLE OF LIFESTYLE FACTORS VS EARLY ORIGINS OF CARDIOVASCULAR DISEASE**

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Introduction: Lifestyle throughout the whole life course makes an important contribution to development of metabolic and cardiovascular disease. Diet, physical activity and smoking are all thought to be whole life determinants of metabolic health and disease. In particular, there is compelling epidemiological evidence that exercise reduces all-cause and cardiovascular mortality, while intervention studies suggest that the
mechanisms responsible include cardiac, vascular, metabolic and autonomic adaptations. Being born pre-term and/or small for gestational age is a well-established risk factor for metabolic and cardiovascular disease. Physical activity has relevance to this relationship from multiple perspectives. Firstly, with regard to physical activity during pregnancy and its effects on birth weight and pre-term delivery and secondly, whether physical activity during pregnancy and/or adulthood in those born small or pre-term can prevent or counteract the detrimental effects of low birth weight is an important clinical and public health question. This is particularly since the prevalence of pre-term birth is rising in many developed countries as a result of increasing maternal age, use of assisted-reproductive techniques as well as advances in neonatal intensive care over the past 40 years which have reduced the limit of fetal viability to around 24 weeks gestation.

Maternal exercise: The importance of maternal lifestyle on fetal health is well recognised. Evidence from epidemiological birth cohort studies suggests that the effects of moderate physical activity during pregnancy on birth weight are small, but reduce the risk of either high or low birth weight infants (Juhl et al., 2010). In contrast, intense exercise in the context of either sport (Gollenberg et al., 2011) or agricultural work (Launer et al., 1990) has been associated with reduced birth weight. Randomised intervention studies are required to substantiate the epidemiology, but are challenging to conduct in humans. Insights from animal studies regarding the effects of maternal exercise on subsequent development of metabolic and cardiovascular disease in offspring will be discussed.

Exercise in later life in individuals born small or preterm: Whether being born small reduces the propensity or ability to exercise as well as the physiological response to exercise and subsequent disease risk has been the subject of increasing investigation in recent decades. While exercise ability in childhood and adulthood is compromised in extremely low birth weight individuals, regular physical activity attenuates many developmental problems. At an epidemiological level, a study using random population sampling in Finnish men indicates the association between birth weight and metabolic disease is lost in fit individuals, and consistently, that the association between low birth weight and metabolic syndrome is accentuated in unfit individuals (Laaksonen et al., 2003). Interestingly, genetics and early habit formation are more likely to influence physical activity and function than birth weight (Hallal et al., 2006). Intervention studies suggest that most cardiometabolic risk factors respond to lifestyle interventions including exercise in a manner which is independent of being small for gestational age, although HOMA-IR response had a small component (4%) related to birth weight (Reinehr et al., 2010). Physiological mechanisms by which exercise may protect early birth weight infants from metabolic failure, restoration of muscle mass, beta-cell mass and function, along with as effects on both aerobic and anaerobic muscle metabolism, including substrate utilisation and mitochondrial function. Vascular and cardiac adaptations are also likely important, but are less well studied.


INTRODUCTION OF A CHROMOSOME 2 QUANTITATIVE TRAIT LOCUS RESTORES ALDOSTERONE REGULATION AND SALT SENSITIVITY IN THE STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT

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Despite significant research efforts, the genetic contribution to salt-sensitivity remains unknown. We have previously identified a quantitative trait locus (QTL) on chromosome 2 that accounts for 20–25 mm Hg of arterial pressure in the stroke-prone spontaneously hypertensive rat (SHRSP). This congenic interval contains a number of candidate genes, including genes involved in aldosterone synthesis. We hypothesised that introduction of this congenic region from the normotensive WKY into the hypertensive SHRSP may restore aldosterone regulation and reduced salt-sensitivity in the SHRSP. Hemodynamic parameters were measured by radiotelemetry from 12–21 weeks of age in WKY, SHRSP and our congenic strain (SP.WKYGla2k) with the congenic interval (10 cM) from the WKY introgressed into the SHRSP background. At 18 weeks of age, rats were treated with water or 1% NaCl for 3 weeks after which in vivo renal function was assessed using clearance techniques and metabolic cation measurements. Plasma and urinary aldosterone was measured using radioimmunoassay and adrenal gene expression was assessed via qRT-PCR. In response to the 1% salt challenge, there was a chromosome 2 dependent increase in systolic blood pressure; increases of WKY: 141.3±3 mmHg to 147.3±3 mmHg, SP.WKYGla2k: 170.7±7 mmHg to 201.4±4 mmHg and SHRSP 194.5±5 mm Hg to 235.6±6 mm Hg (P<0.001). SHRSP also had a greater sodium excretion (194±20 mg/24 h vs 119±10 mg/24 h respectively, P<0.0005) and greater plasma aldosterone concentration compared to WKY (0.86±0.17 nmol/L vs 0.53±0.05 nmol/L respectively, P<0.04). Furthermore, SHRS has significantly greater arterial 3H-hyd and 3J-hyd mRNA expression compared to WKY, genes located in the chromosome 2 congenic interval involved in the aldosterone synthesis pathway. There was no difference in exon parameters or adrenal mRNA gene expression between SP.WKYGla2k and WKY suggesting that this congenic region small aldosterone regulation and sodium handling to WKY levels. Our findings suggest that the introgression of the small chromosome 2 congenic interval from the WKY on the hypertensive background is associated with restored aldosterone regulation and is sufficient to reduce salt-sensitive hypertension.

THE INFLUENCE OF SPIRONOLACTONE ON RENAL FUNCTION AND CARDIOVASCULAR DISEASE IN POLYCYSTIC KIDNEY DISEASE

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Polycystic kidney disease (PKD) is a progressive genetic disorder ultimately leading to renal failure, associated with hypertension and heart disease. We have shown previously in the Lewis polycystic kidney (LPK) rodent model of autosomal-recessive PKD that aldosterone levels increase in association with deteriorating renal function. In addition to its direct effects on renal sodium reabsorption, aldosterone has been shown to cause renal and cardiovascular injury, and may therefore be a key factor contributing to disease progression. We have therefore examined the hypothesis that inhibition of aldosterone with Spironolactone will reduce the severity of cardiovascular disease and improve renal function in PKD. Spironolactone (20mg/kg/day p/o) was administered in water to LPK and the control Lewis strain from 4–12 weeks of age. Animals were divided into 8 groups according to strain, gender and treatment (n=14 LPK, n=16 Lewis). Systolic blood pressure was measured fortuitously via tail-cuff method and urine was collected in a metabolic cage at age 12 weeks for urinary 24hr volume and protein:creatinine ratio analysis. At 12 weeks the animals were weighed and euthanized, trunk blood collected, and the heart dissected and weighed. In the LPK, blood pressure increased over the 6–12 week time frame. Spironolactone had a significant effect in reducing blood pressure in the female LPK only (179±3 systolic vs 209±3 mm Hg, P<0.03). LPK untreated animals had a higher heart weight; body weight ratio (0.54±0.02) compared to the treated LPK group (0.52±0.03, P<0.047), both of whom had significantly larger hearts than the Lewis (P<0.001). The left ventricle/heart weight ratio indicated LVH in both LPK groups (P<0.001), which was not influenced by spironolactone treatment. Urine creatinine: protein ratio improved significantly in the LPK treated group, such that it was no longer different to Lewis animals. In summary, spironolactone had a beneficial effect on renal function in the LPK model but did not significantly impact cardiac status. Additional studies will examine the degree of cardiac fibrosis in the different treatment groups. The outcomes of this study will be of benefit to determining optimal treatment strategies for patients with PKD.
**DIRECT AT2 RECEPTOR STIMULATION SUPPRESSES CIRCULATING T CELL ACTIVATION AND VASCULAR INFLATION IN ANGIOTENSIN II-INDUCED HYPERTENSION**

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The adaptive immune system, via the role of T cells, has recently been implicated in the development of experimental hypertension. Since T cells express functional components of the renin-angiotensin system (RAS) including the AT2 receptor (AT2R), and direct AT2R stimulation has been shown to exert anti-inflammatory actions, this study investigated whether an AT2R agonist, CGP42112, could prevent T cell activation and vascular inflammation associated with angiotension (Ang) II-induced hypertension. C57BL/6J mice received 2-week treatment of either vehicle, Ang II (0.7 mg/kg/day), Ang II + candesartan cilexitil (AT1R antagonist; 1 mg/kg/day), Ang II + CGP42112 (1.4 mg/kg/day). Blood, kidney and aorta were harvested and processed for flow cytometric analysis for markers of T cell activation (CD44+ and CD69). As expected, Ang II significantly increased systemic blood pressure (Ang II: 154±4 mmHg vs Vehicle: 107±2 mm Hg, P<0.001, 2-way ANOVA) and was not altered by CGP42112 treatment, but was abolished by AT1R blockade treatment. Consistent with current literature, Ang II increased circulating CD4+ T-helper cell activation as surface expression of early T cell activation marker, CD69 (Vehicle: 4.7±0.6 % Vs Ang II 8.4±0.7 %, P<0.05; 1 way ANOVA), and effector T cell marker, CD44+ (Vehicle: 12.4±5.9 % Vs Ang II 20.8±1.4 %, P<0.001; 1 way ANOVA), was significantly elevated compared to vehicle-treated mice. Interestingly, CGP42112 reversed Ang II induced CD4+ T-helper cell activation to a greater extent than AT1R blockade, but also increased cytoxic T cell (CD3+CD8+) expression (Ang II: 3.9±0.4 % Vs Ang II+CGP: 7.2±1.4 %, P<0.01). Moreover, Ang II-induced leukocyte (CD45+) and T cell (CD3+) infiltration into the aorta and kidney was significantly attenuated in mice treated with CGP42112 or candesartan cilexitil. Collectively, our findings demonstrate AT2R-evoked suppression of the adaptive immune system in hypertension, and may represent a novel strategy for the treatment of inflammation associated with cardiovascular disease.

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**QUALITY OF LIFE IN PATIENTS WITH BILATERAL PRIMARY ALDOSTERONISM BEFORE AND DURING TREATMENT WITH SPIRONOLACTONE AND/OR AMILORIDE, INCLUDING A COMPARISON WITH OUR PREVIOUSLY PUBLISHED RESULTS IN THOSE WITH UNILATERAL DISEASE TREATED SURGICALLY**


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Measurement of quality of life (QOL) allows assessment of the impact of a disease or treatment from the patient’s perspective, including need for social, emotional, or physical support. We are not aware of any published QOL assessment in patients with bilateral primary aldosteronism (BPA), before or after commencing medical treatment (MT) with spironolactone and/or amiloride. Using the internationally validated Medical Outcomes Study Short Form 36 General Health Survey (SF-36), QOL was assessed in 21 patients with BPA at baseline (time of diagnosis), and at 3 and 6 months after commencing MT. OSLQ scores at baseline were compared with published normative values for the Australian population. The results of the current study were compared with those from our previous study showing reduced QOL in patients with unilateral primary aldosteronism (UPA) with normalization by 3 months after unilateral laparoscopic adrenalectomy. Compared with the general population, patients with BPA showed significant reduction (P<0.01) in four QOL domains—physical functioning, role limitations due to physical health problems, general health perceptions, and vitality. After 6 months (but not 3 months) of MT, statistically significant (P<0.05) improvements were detected in all these domains of QOL. When compared with patients with UPA treated surgically, scores were significantly (P<0.05) lower at 3 months for five domains (role limitations due to physical health, general health, role limitations due to emotional health, mental health, and vitality) but at 6 months for only one domain (role limitations due to emotional problems). Subnormal OSLQ scores were improved after 6 months of MT in 21 patients with BPA, but more slowly and to a lesser degree than surgical treatment had previously been shown to improve QOL scores in 22 patients with UPA.

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**REAL-TIME BLOOD PRESSURE MONITORING DURING HAEMODIALYSIS IDENTIFIES UNRECOGNISED HAEMODYNAMIC INSTABILITY, ESPECIALLY IN DIABETIC SUBJECTS**

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Haemodialysis (HD) is an important therapy for managing the solute, fluid and blood pressure (BP) problems in renal failure but the cardiovascular stress of an HD treatment itself can cause problematic acute changes in BP. In standard clinical practice, BP is measured by arm-cuff sphygmomanometer at hourly intervals throughout HD. By convention, intradialytic hypotension is defined as a symptomatic absolute BP drop ≥20 mm Hg. We used the Finometer Midi system to obtain real-time, beat-to-beat measures of systolic BP during standard 4-hour HD in 15 subjects (3 female, mean age 64 years) with no clinical history of intradialytic instability. Four subjects were diabetic and 14 were routinely prescribed antihypertensive medication. Pre-dialysis plasma renin, aldosterone and NT-proBNP levels were obtained in all patients. BP variability, and the magnitude and direction of swings, were quantitatively assessed using a continuous rate-of-change momentum indicator. Variation was determined against a moving baseline defined by sequential interval units of 500 heartbeats. Significant BP changes were defined as ≥20% above or below baseline. Comparing the coincident measures of arm-cuff and Finapres recordings revealed strong concordance with a mean difference of 0.6 mm Hg (SD 6 mm Hg). Sudden discrete fluctuations in BP were seen on 184 separate occasions across the 15 HD sessions. Rises in BP (n=108) were more common than sudden falls (n=76). Fluctuations were more common in diabetics compared with non-diabetics (mean 24 and 8 events/HD respectively, P<0.001). Two patients, both diabetic, developed clinical symptoms associated with a BP drop that responded to brief cessation of ultrafiltration. The overall frequency and magnitude of BP variability was not predicted by the use of antihypertensives.
FACTORS ASSOCIATED WITH BLOOD PRESSURE VARIABILITY AND ITS EFFECT ON CARDIO-VASCULAR OUTCOMES IN ELDERLY HYPERTENSIVE PATIENTS: FINDINGS FROM THE 2ND AUSTRALIAN NATIONAL BLOOD PRESSURE STUDY

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Systolic blood pressure (SBP) variability is a strong predictor of cardiovascular events, especially stroke. High SBP is common amongst the elderly and is difficult to control. Little is known about the level of SBP variability in the elderly in Australia and, more importantly, the factors associated with SBP variability and its impact on clinical outcomes. The aim of the current analysis is to identify factors associated with visit-to-visit variability in SBP and its effect on cardiovascular events in elderly hypertensive patients participating in the 2nd Australian National Blood Pressure (ANBP2) study. ANBP2 was a prospective, open label study with blinded assessment of endpoints designed trial conducted in 6083 hypertensive patients aged 65 to 84 years. Patients were randomised to an ACE-inhibitor or diuretic-based blood pressure lowering regimen and were followed up for a median 4.1 years. General practitioners (GPs) or study nurses recorded BP for each patient during their follow-up visits. The mean (SD) number of visits per patient was 10.5 (±3) (n=8634, range 1–24 visits). In this analysis, we defined the visit-to-visit SBP variability of an individual as the standard deviation in SBP between visits. SBP variability was then categorised into quartiles. Multiple logistic regression was used to identify the factors associated with SBP variability and then Cox-proportional hazard models were used to explore the relationship between CVD events and SBP variability. Greater visit-to-visit SBP variability was associated with the following clinical factors: age, pulse pressure, treatment allocation and the use of two or more blood pressure lowering drugs during in-study. In our study cohort the on treatment visit-to-visit SBP variability was a strong predictor for CVD events. The hazard ratio (95% confidence interval) of SBP variability for myocardial infarction was 1.69 (1.19–2.31) for stroke 1.53 (1.15–2.03), and for heart failure 1.80 (1.29–2.52) after adjusting for sex, age, treatment allocation and in-study use of two or more blood pressure lowering drugs. SBP variability, in addition to the absolute level of blood pressure, may be an alternate target for cardiovascular risk reduction in elderly patients managed for hypertension.

THE EFFECTS OF A 6-MONTH COMMUNITY-BASED PHYSICAL ACTIVITY PROGRAM ON THE BLOOD PRESSURE OF OLDER MEN AND WOMEN

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We aimed to evaluate the effects of a 6-month supervised walking versus a self-managed physical activity (PA) program on the home blood pressure (BP) levels of 60–80 year olds. Healthy sedentary women (n=204) and men (n=82) were recruited to 12 recreation centres in the Perth metropolitan area. The centres were stratified according to socio-economic status (SES) and randomly assigned to the supervised walk or self-managed program. Participants were asked to complete 150 minutes/week of moderate physical activity. They recorded their own home BP using a digital BP monitor for 4 measurements 2 minutes apart on 3 occasions during a week at baseline and after 6 months. The number of PA sessions completed was recorded by participants. Weight, fitness and lifestyle were evaluated at baseline and 6 months. Models for predicting change in weight and change in blood pressure were adjusted for SES and corrected for within-centre correlation using centre as a random effect. There was no significant difference between groups in change in weight or change in BP after 6 months. Change in weight was predicted by the number of exercise sessions completed, divided into tertiles, with a significant difference (P=0.03) between the lowest (2.4 kg·m⁻²·yr⁻¹) and highest (−0.2 kg·m⁻²·yr⁻¹) tertile. Change in weight predicted change in SBP (P=0.005). Change in SBP differed significantly between the highest and lowest exercise tertile (P=0.033), SBP increased (2.7 mm Hg ≥2.0 mm Hg) in the lowest tertile and decreased in the highest tertile (−1.4 mm Hg). Similarly, change in weight predicted change in DBP (P=0.033) which differed significantly (P=0.034) between the lowest exercise tertile (0.6 mm Hg ≥1.0 mm Hg) and the highest (−1.7 mm Hg). This community-based program was effective in increasing the PA levels of older adults with higher levels predicting change in weight and this weight change predicting change in home BP. Higher levels of PA resulted in greater improvements in BP. These results demonstrate that even with the modest amounts of moderate intensity walking achieved by participants higher amounts of PA lead to greater health benefits.

METABOLIC PROFILE OF SCHLAGER HYPERTENSIVE MICE

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Hypertrophic regions have been shown to have greater neuronal activation in the hypertensive BPH/2J mice compared with normotensive BPH/3J mice. Gene array studies performed in hypothalamic tissue from Schlaguer mice show the hypocretin gene is upregulated in BPH/2J compared with BPH/3J mice. Hypocretin or orexin as it is alternatively known is shown to be involved in cardiovascular control, sympathetic outflow, stress response, activity and metabolic state and energy balance. There is a noticeable association between the orexin upregulation in BPH/2J mice and previously reported phenotype abnormalities including hypertension, sympathetic over-activity, exaggerated stress response and greater locomotor activity levels. As it is therefore possible that the metabolic profile of BPH/2J mice may also be irregular. To determine whether BPH/2J mice have an abnormal metabolic profile, 48 BPH/3J and 4 BPH/2J mice were placed in a Comprehensive Laboratory Animal Monitoring System (CLAMS) for 24 hrs. This system measures food intake, rate of oxygen consumption (VO2) and carbon dioxide (VCO2) production and total HRV (in Hz) automatically using EchoMR. Mice were assessed at 10 and 23 weeks of age to assess changes during development. BPV/2J mice showed greater VO2 and VCO2 compared with BPH/3J mice at both ages (P<0.0001), yet this declined with age in both species (P<0.0001). Heat production was higher in BPH/2J mice at 10 (P<0.0001) and 23 (P<0.003) weeks of age. Total activity level was greater in BPH/2J compared with BPH/3J at both ages (P<0.0001) indicating greater energy demand but this only declined with age in the BPH/3J, whilst the BPH/2J activity remained higher. There was a markedly greater food consumption in 10 week old BPH/2J mice compared to BPH/3J (P=0.0001) which disappeared with age (P=0.4). Yet body weight (BW) was consistently lower in BPH/2J compared with BPH/3J mice at both ages (P=0.003). However when expressed as percentage of BW, lean mass, fat mass and total water content were not different between strains, suggesting no abnormality in body composition. These results show BPH/2J mice have greater activity related energy demands at both ages, yet food intake, VO2, VCO2 decline with age. This may indicate a change in energy efficacy with age.

CROSS COMPARISON OF HEART RATE VARIABILITY AND BAROREFLEX SENSITIVITY IN RODENT MODELS OF DISEASE

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Heart rate variability (HRV) and/or baroreflex sensitivity (BRS) are reduced in many disease states, including hypertension, kidney disease and depression, and may signify an increased risk of cardiac mortality. It is not well described if reduced levels of HRV and/or BRS are comparable between different disease states. We compared HRV and BRS in conscious telemetry and urethane anaesthetised rodent models of hypertension (Spontaneously Hypertensive Rat [SHR, n=14], kidney disease [Lewis Polycystic Kidney [LPK, n=18] and depression [Flinders Sensitive Line [FSL, n=11]), and their respective genetic controls (Wistar Kyoto [WKY, n=12], Lewis n=14) and Sprague Dawley [SD, n=12], to address the following questions: (1) Are HRV and BRS reduced in these rodent models of disease compared with their controls? (2) Do different disease models have similarly reduced HRV and BRS; (3) Is HRV and BRS similar among control strains; and (4) What effect does anaesthesia have. Our results indicate: (1) Only the LPK and FSL exhibited reduced HRV or BRS compared with their control, WKY and SD, respectively. (2) LPK exhibited similar HRV and BRS compared with the anaesthetised conditions, while the FSL exhibited reduced total and high frequency HRV power and BRS. BRS was reduced in conscious LPK and FSL; (2) HRV was similar between the SHR, LPK and FSL under anaesthetised and conscious conditions with the exception that high frequency power was lower in conscious FSL. BRS, however, differed among the diseased strains with BRS lowest in the LPK under anaesthesia and in the FSL under conscious conditions; (3) HRV and BRS parameters were generally similar among the control strains with the only notable difference being an increase in BRS in the WKY under conscious conditions and (4) Urethane anaesthetia had suppressive effects on HRV in all strains examined and reduced BRS in the LPK, WKY and Lewis. These results indicate that reductions in HRV can be observed in rodent models of disease under anaesthetised conditions, likely due to the suppressive actions of urethane on HRV that may be accentuated by disease. Reductions in BRS, however, could be observed irrespective of the anaesthetised conscious state and may be a more reliable index to assess cardiac risk in rodent models of disease.

RELATIONSHIP BETWEEN ABRANT CARDIAC MICRONRNA EXPRESSION IN HERITABLE POLYGENIC CARDIAC HYPERTROPHY

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From soon after birth the Hypertrophic Heart Rat (HHr) normotensive model of human polygenic cardiac hypertrophy has a reduced complement of terminally differentiated cardiomycocytes, leading to hypertrophy, ventricular premature depolarisation and a dilated hypertrophic heart. The regulatory mechanisms that govern these processes are poorly understood. Given the emerging role of the short non-coding microRNAs (miRNAs) in influencing genetic pathways and hence disease, we searched for miRNAs that were regulated during the neonatal period in this strain compared to the normotensive Heart Rat (HR). Nontoxic RNA was purified from the hearts on postnatal day 2 HHR (n=4 males, n=4 females) and NR (n=4 males, n=4 females) using MiRNeasy kit (Qiagen, Inc.). Microarray analysis was performed using Agilent Rat miRNA Microarray Kit
Release 16.0. Bioinformatic analysis revealed a number of miRNAs that were up-regulated or down-regulated in cardiac ventricles from the HHR at 2 days of age. A total of 31 miRNAs were differentially regulated during day 2. 16 miRNAs were up-regulated while 15 were down-regulated. Six of these miRNAs showed a statistically significant interaction with sex. Many of these miRNAs are known to be involved in cell growth and apoptosis. One of the most robustly down-regulated miRNAs between HHR and NHR was miR-466 (P<0.001, FDR 0.01) with expression levels almost 60-fold lower in day 2 ventricles of HHR relative to NHR. The down-regulation of miR-466 is known to hinder the inhibition of several anti-apoptotic genes in unison. We also found miR-378 to be significantly up-regulated (P=0.001, FDR 0.01), miR-378 is known to enhance cell survival and angiogenesis through repression of the expression of two tumour suppressors, Sufu and_Fus1. These findings suggest that dysregulation of various microRNAs during the neonatal period may be an important regulatory mechanism governing cardiac myocyte differentiation in the HHR.

**ALISKIREN REDUCES MYOCARDIAL INFARCT SIZE IN SPRAGUE DAWLEY RATS AT A DOSE THAT DOES NOT REDUCE CIRCULATING OR CARDIAC ANGIOTENSIN II LEVELS**

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Aliskiren is a renin inhibitor recently approved for the treatment of hypertension. We previously reported that 10 mg/kg/day aliskiren increases bradykinin and tissue kallikrein mRNA levels in the heart of Sprague Dawley rats independently of change in circulating and cardiac angiotensin levels. To investigate whether aliskiren is cardioprotective independent of change in circulating and cardiac angiotensin levels, we administered vehicle, aliskiren (10 mg/kg/day by subcutaneous minipump), the angiotensin II type 1 receptor blocker valsartan (30 mg/kg/day by gavage) and the combination of aliskiren and valsartan to female Sprague Dawley rats. After 4 weeks of treatment, the rats were subjected to ischaemia-reperfusion injury of the heart produced by 30 min occlusion of the left anterior descending coronary artery followed by 120 min reperfusion. None of the treatments affected body weight. Neither aliskiren nor valsartan influenced systolic blood pressure (SBP) as assessed by tail cuff plethysmography, whereas the combination of aliskiren and valsartan reduced SBP by 12 mm Hg (P<0.01). Mean arterial blood pressure of the anaesthetised rats did not differ between the 4 groups during ischaemia-reperfusion. Myocardial infarct size, expressed as the percentage of area at risk, was 40.3% (mean±SEM, n=7–11) in vehicle-treated rats. Myocardial infarct size was reduced similarly by all 3 treatments; aliskiren: 24.5% (n=7, P=0.035, Dunnett’s t-test); valsartan: 25.6% (n=6, P=0.048); aliskiren plus valsartan: 22.3% (n=8, P=0.012). We conclude that aliskiren and valsartan produced similar cardioprotection, without further cardioprotection by the combination. Together with our previous studies, these results suggest that aliskiren is cardioprotective independent of change in circulating and cardiac angiotensin II levels, and its cardioprotection may be dependent on the associated increase in cardiac bradykinin and tissue kallikrein expression.

**COMPARISON OF DIRECT MEASUREMENTS OF BLOOD PRESSURE (BP), HEART RATE (HR) AND RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) IN CONSCIOUS RABBITS**

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The methodological improvements in measuring biological parameters such as BP, HR and sympathetic nerve activity (SNA) have given a great impact on the investigation of hypertension as well as blood pressure related disease. The aim of this study was to compare the direct method using arterial catheterization BP, HR and SNA in rabbits with radio-telemetric measurement in consciously moving laboratory animals and conscious constrained rabbits in a BP measurement box. Male New Zealand white rabbits (2.6 to 3.1 kg) were housed under controlled light (6:00 AM to 6:00 PM) and temperature (22.2±0.2°C) conditions. All rabbits received a radio-telemetry implant to monitor BP, HR and SNA for 3 weeks. All experiments were conducted at least 1 week after renal electrode surgery. Pulsatile arterial blood pressure was measured and RSNA between 50 and 2 kHz was rectified and integrated using an integrator filter with a 20 ms time constant. BP, HR and integrated RSNA were digitized and averaged over 2 s periods. RSNA was normalized in each rabbit relative to the maximum 2 s of RSNA evoked by 50 mg cigarette smoke at the start of each experiment. Maximum RSNA was taken to equal 100 normalized units. There were no significant between-group differences in the maximal RSNA response to smoke before normalization. BP (69.2±0.6 and 70.7±0.7 mm Hg in caged and lab constrained animals, respectively) and RSNA (7.9±1.0 and 7.5±0.8 in caged and lab constrained animals, respectively) were not different to between caged and lab constrained rabbits throughout the experimental period. HR (181.2±3.9 and 168.4±3.7 beat/min, P=0.0002) was significantly higher in caged rabbits when compared to constrained rabbits. In conclusion, our findings clearly demonstrate that BP and RSNA measurements are consistent in both caged housed rabbits and lab placed constrained rabbits hence, showing placing the rabbits in a measurement box do not implement stress to the animals.

**DIFFERENTIAL CHANGES IN LARGE ARTERY HAEMODYNAMICS FOLLOWING SYMPATHETIC BLOCK IN NORMOTENSIVE AND HYPERTENSIVE RATS**

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Arterial stiffness is a predictor of cardiovascular disease and all-cause mortality. Arteries stiffen with age and as a result of lifestyle influences and some disease states which places a greater load upon the heart. The stiffness of arteries is determined by the structure of the artery wall and the tension applied by smooth muscle within the artery wall. Smooth muscle tone is controlled by many factors, one of which is sympathetic nerve inputs. This study identifies the extent to which sympathetic activity affects large arterial haemodynamics in both the normotensive and hypertensive condition. Following removal of sympathetic nerve activity via injection of hexamethonium (20 mg/kg) normotensive rats (WKY, n=7) showed a significant increase in aortic diameter, compliance and distensibility at operating blood pressure (100 mm Hg). However, within hypertensive rats (SHR, n=9), the increase in aortic diameter following injection of hexamethonium was not accompanied by a change in any measured stiffness parameters at operating blood pressure (150 mm Hg). These results reflect both the contribution of sympathetic nerve activity to arterial haemodynamics as well as the differential influence of sympathetic input on arteries that have developed under different pressure states. These results should help contribute to the understanding of the relationship between hypertension, arterial stiffness and sympathetic nerve activity in man.

**INFLUENCES OF NEUROMUSCULAR BLOCKERS ON VAGAL CONTROL OF HEART RATE**

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Neuromuscular blocking drugs (NmBs) inhibit nicotinic acetylcholine receptors at the skeletal neuromuscular junction. These drugs are used during surgery to facilitate mechanical ventilation and the application of continuous paralysis. Nicotinic acetylcholine receptors are also located within autonomic ganglia and NmBs may therefore influence both sympathetic and parasympathetic (vagal) control of the cardiovascular system. Given the use of these drugs in research to facilitate the study of the cardiovascular system, our objective was to examine 3 different NmBs: pancuronium and rocuronium (benzylisoquinolinoid nondepolarizing NmBs) and cisatracurium (a benzyloquinoline nondepolarizing NmB) on vagal control of heart rate (HR). Adult urethane (1.3 g/kg i.p) anaesthetised Lewis rats (n=6 rats per group, n=15 rats per group) were placed on a sensor belt and instrumented to record blood pressure and HR and a sympathetic β-blocker atenolol (1 mg/kg i.v) was administered to inhibit sympathetic control of HR. Rats then received (i) pancuronium (bolus dose (2 mg/kg) then continuous infusion (1 mg/kg/hr)), (ii) rocuronium (bolus dose (12 mg/kg) and continuous infusion (10 mg/kg/hr)) or (iii) cisatracurium (6 mg/kg) followed by continuous infusion (6 mg/kg/hr) and changes in HR, mean arterial pressure (MAP) and baroreceptor reflex sensitivity (BRS), measured as a change in HR evoked in response to phenylephrine (50 μg/kg) increase in MAP, assessed. None of these NMBS altered MAP. Cisatracurium and rocuronium had no effect on HR, whereas pancuronium increased HR (276.9±8.3% vs 365.3±12.9%, P<0.05). BRS was reduced by pancuronium and rocuronium but not cisatracurium. Furthermore, when neuromuscular blockade was achieved with pancuronium, BRS was lower when pancuronium or rocuronium were used (0.25±0.02 vs 0.68±0.11 and 0.69±0.10 pm/mg Hg, respectively, P<0.05). These results indicate that cisatracurium has the least inhibitory effect on vagal control of HR. Future studies will investigate if the cardiac vagal protection effects of cisatracurium are preserved under different anaesthetic conditions, therefore providing information as to the best anaesthetic-NMB combination in which to investigate vagal control of HR.
REDOX MODULATION OF THE ASCENDING NORADRENERGIC SYSTEM: A ROLE IN THE CARDIOVASCULAR RESPONSES TO EMOTIONAL STRESS

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Altered activity in the brain noradrenergic (NA) system has long been recognized as a key element of many aspects of pathological anxiety. More recently, reactive oxygen species, such as the superoxide anion (O2–), have been suggested as regulators of fear-related cardiovascular outcomes. In the present study we examined whether oxidative stress and the redox state of the brain NA system play a role in regulating the cardiovascular response to fear-related stimuli in normotensive rat strains. We found that lentinus-mediated overexpression of superoxide dismutase 1 (SOD1) in the A1/C1 cell group increases blood pressure response to conditioned anxiety stimuli significantly over control animals (26±1 vs 21±1 mm Hg respectively, P<0.02, n=6). Conversely, cardiovascular arousal associated with routine daily activities occurring while in their home cages was similar between SOD1 and control groups (16±3 and 14±2.2 mm Hg, respectively). Baseline blood pressure was also similar between both the SOD1 and control animals (97±1.9 and 95±2.3 respectively). Baseline arterial blood chemistry indicates that SOD1 vector expression can reduce O2– levels by up to 60%. The increased cardiovascular reactivity may relate (at least in part) to elevated emotional reactivity, as SOD1-transduced animals displayed increased behavioural indices of anxiety, including freezing, and ultrasonic vocalization, and reduced general activity levels. These data suggest that the redox regulation of the ascending NA system may play an important role in the cardiovascular response to emotional stressors but not in tonic maintenance of blood pressure.

POST-ISCHEMIC CONTRACTILE RECOVERY OF HYPERTROPHIED FEMALE CARDIOMYOCYTES IS DETERMINED BY MECHANISMS INDEPENDENT OF Ca2+-TRANSIENT RESTORATION

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Clinically, male/female differences in hypertrophic pathoogy and post-myocardial infarction outcomes have been identified. At the cellular level, altered contractile performance and disturbances in Ca2+ handling are associated with both hypertension and ischemia, but the underlying mechanisms for sex differences remain unknown. This study aimed to assess contractile and Ca2+- handling responses in male/female hypertrophied cardiomyocytes subject to a simulated in vitro ischemic insult. We utilized the Hypertrophic Heart Rat (HHR) strain, a model of normotensive, primary cardiac hypertrophy, for the investigation of differential perturbations in sympathetic outflow to distinct effector organs. Furthermore, we investigated whether sex-specific interventions exhibiting similar Ca transient amplitudes. This indicates male/female myocytes respond

response to L-NAME was similar in WT, HET-2K and HET-1K mice (=17.4±0.5 mm Hg, +16.2±1.2 mm Hg, +17.4±1.8 mm Hg respectively), MAP of WT mice remained stable over the 7day period. However, the initial rise in MAP of HET-2K and HET-1K mice was gradually attenuated over the following 6 days such that at Day 7 the rise in MAP was greatest in WT (=15.3±1.1mm Hg), followed by HET-2K (=11.1±1.7mm Hg) and least in HET-1K mice (=7.6±2.9mm Hg, P<0.05). In conclusion, at 30 weeks of age GDNF HET mice appear less dependent on NO in the control of MAP compared to WT mice. These data suggest that other vasodilatory pathways may be upregulated in neprhin deficient GDNF HET mice when NO bioavailability is limited and may contribute to the preservation of renal health and normal blood pressure in these mice.

BAROREFLEX CONTROL OF THE RENAL SYMPATHETIC NERVE IN AUTOSOMATIC RECESSIVE CYSTIC KIDNEY DISEASE

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Chronic kidney disease is associated with cardiovascular autonomic dysfunction. In a rat model of autosomal recessive cystic kidney disease, we have previously shown impaired baroreflex control of renal sympathetic nerve activity (RSNA) and impaired renal sympathetic nerve activity (RSNA) in response to phenylephrine (10 –50 μg/kg) and sodium nitroprusside (50–70 μg/kg)-induced alterations in mean arterial pressure (MAP) were recorded. The MAP-nerve activity relationship was characterized using a four-parameter sigmoid regression. Compared to Lewis control, LP rats had significantly higher resting heart rate (HR, 351.7±4 vs 414.1±10 beats per minute, P<0.0001), systolic blood pressure (SBP, 113.3±4 vs 174.8±8 mm Hg, P<0.0001) and RSNA (3.5±0.5 vs 6.6±0.8 μVL, P<0.001) but comparable ADNA (0.9±0.2 vs 1.0±0.3 μVL). The ADNA barocurve was comparable in LP and Lewis rats but shifted rightward in the LPKs, suggesting an intact sensory afferent pathway that is operational at higher resting MAP. The RSNA baroreflex curve was shifted to the right towards higher MAP range; but there was a marked reduction in the gain (P=0.0003) and the magnitude of reflex sympathoinhibition (P<0.005), indicating impaired baroreflex control of RSNA. We conclude that unlike baroreflex control of the sympathetic nerve, baroreflex control of RSNA is impaired in the LPK model, indicative of differential perturbations in sympathovagal autonomic function. Furthermore, our results suggest that impaired sympathetic baroreflexes are due to altered central and/or effrent mechanisms rather than a defect in the sensory afferent pathway of the reflex.

THE PLEOTROPHIC EFFECT OF LEPTIN AND LEPTIN RECEPTOR GENES ON BMI AND AGGRESSION SCORES IN ADOLESCENTS

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Obese children are more likely to become obese adults and are at a greater risk of developing cardiovascular disease. Emotions have been linked to obesity in childhood; however the mechanism underlying this co-occurrence remains unclear. Leptin is a key regulator of body weight and has been implicated in the regulation of emotions. This study aimed to investigate whether single nucleotide polymorphisms (SNPs) within the leptin (LEP) and leptin receptor (LEPR) genes underlie the co-occurrence of aggressive behaviour scores and body mass index (BMI) in adolescents. The study used data on 1064 participants from the Western Australian Pregnancy Cohort (Raine) Study at the 14 year survey. Tagged SNPs for LEP and LEPR were identified from HapMap Phase II (CEU) data and genotyped. The adolescent’s weight and height were assessed by trained assessors and BMI was calculated. Aggressive behaviour scores were derived from the Child Behavior Checklist/4–18. A linear regression model was used to examine cross-sectional associations between BMI (outcome) and tagged SNPs within LEP and LEPR. Mixed distribution models were used to examine associations between aggressive behaviour score (outcome) and SNPs within LEP and LEPR. Analyses were stratified by gender. After adjusting for multiple testing, only SNP rs9436737 within LEP was independently associated with both BMI and aggressive behaviour score in adolescent boys but not girls. Boys with one or more copies of the G allele had higher BMI (20.2 kg/m2 vs. 19.6 kg/m2, P<0.001) and higher aggressive behaviour score (2.4 vs. 2.3, P=0.0006) compared to boys without a copy. Mutual adjustment suggested that this SNP was associated with each outcome independently of the other. SNPs within LEPR may underlie the co-occurrence of aggression and BMI in adolescent boys but not girls. Our findings, if replicated, may have implications for understanding the mechanism through which LEPR may act to independently effect obesity and aggression.
TECHNICAL DEVELOPMENT OF A DIABETES MEDICATION USING THE HUMAN RUSSIAN SPARROW AS A MODEL SYSTEM

H.J. Oosthuizen

This paper presents a technical development of a diabetes medication using the human Russian sparrow as a model system. The study involved the use of this model organism to evaluate the effectiveness of various compounds in the treatment of diabetes. The research aimed to identify potential diabetes medications that can be used in human patients. The findings of the study suggest that the Russian sparrow is a suitable model for the development and testing of diabetes medications, with promising results indicating potential therapeutic benefits.
ASSOCIATION OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH TYPE II DIABETES AFTER ACUTE CORONARY SYNDROME

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Patients with type II diabetes (T2D) with a previous history of acute coronary syndrome (ACS) are at increased risk for subsequent major cardiovascular events compared with patients without T2D. Since endothelial function plays a critical role in vascular health, particularly in the context of diabetes, this study examined whether standard clinical care was effective in normalising endothelial function in patients with T2D, 2 to 6 months post ACS, compared with sex- and age-matched healthy controls. Thirty one patients (15 T2D; 16 controls) were recruited for a single study visit to the Alfred Heart Hospital Centre. T2D+ ACS patients on standard clinical care exhibited residual cardiovascular risk profiles such as increased body mass index, glucose levels and triglycerides and lower HDL levels (n = 15–16; P < 0.01). However, total cholesterol, LDL cholesterol and triglycerides were well controlled and no significant differences were observed in the antiplatelet cocktail (38.3 ± 2.5 vs 39.5 ± 2.7; P = 0.17 vs 2.4 vs 3.3 ± 0.33; respectively; n = 15–16; P = 0.04). Endothelial microparticle (EMP) numbers have also been shown to increase with SSBP correlated with endothelial dysfunction in T2D patients in the current study. EMP (CD34+, CD41+) numbers, measured by flow cytometric analysis, were reduced by 40% in the patient cohort (P = 0.019) while platelet-derived microparticles (PMPs) were unchanged (P = 0.05). This may be due to the medications received. We conclude that patients with T2D and less than 6 months post ACS exhibit residual cardiovascular risk factors including endothelial dysfunction despite being on standard clinical care.

ARTERIAL STIFFNESS IS RELATED TO BLOOD PRESSURE LEVEL AND BLOOD PRESSURE SUTURE RESISTANCE IN HYPERTENSIVE PATIENTS

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1Neurovascular Hypertension & Kidney Disease Laboratory, Baker ID Heart & Diabetes Institute; 2Department of Hypertension and Diabetology, Medical University of Gdansk, Poland; 3Heart Centre Alfred Hospital; 4Neuropharmacology Laboratory, Baker ID Heart & Diabetes Institute. Blood pressure variability is a strong independent predictor of hypertension-related cardiovascular events. The relationship between circadian blood pressure variations and endothelial function is unknown. This study examined whether arterial stiffness and/or endothelial dysfunction influence blood pressure profile in resistant hypertension. We measured office seated blood pressure (BP) and ambulatory BP in 46 patients (33 males) with resistant hypertension (age 61 ± 11 years, BMI 31 ± 6 kg/m2; mean ± SD). Arterial stiffness was measured by the peripheral arterial tone (PAT) signal obtained via finger tiptonometry derived augmentation index (AII); endothelial function was assessed by digital pulse amplitude in response to reactive hyperemia (HAT-510 RAT) using the EndoPAT 2000 system. BP variability was assessed as the standard deviation from both office and ambulatory BP. Morning SBP power surges was defined as the rate of SBP rise multiplied by the amplitude. Office-seated BP averaged 168 ± 18/89 ± 15 mm Hg despite the use of an average of 5.1 ± 1.9 antihypertensive drugs. Mean 24-h daytime systolic (SBP) was 150 ± 11 mm Hg, night-time 138 ± 22 mm Hg; mean daytime diastolic (DBP) 85 ± 14 mm Hg, night-time 76 ± 15 mm Hg. SBP load averaged 76 ± 1 ± 30 ± 3.5 %. AI 30 ± 23 %. PAT ratio 1.7 ± 7.3. A higher AI was significantly related to greater variability of office-seated (r = 0.34; P = 0.02), ambulatory day-time (r = 0.38; P = 0.01) and nighttime (r = 0.39; P = 0.01) SBP. Arterial stiffness was significantly associated with morning SBP power surge (r = 0.38; P = 0.01). Increased nocturnal SBP and SBP load were significantly associated with impaired peripheral vasodilator response (r = 0.43; P = 0.005; r = 0.32; P = 0.02). Arterial stiffness was higher in females than in males (44.6 ± 20.5 vs. 18.4 ± 17.5 %; P < 0.002) when matched for age, BMI and BP. Uncontrolled blood pressure and altered BP variability evident in patients with resistant hypertension is associated with structural (increased arterial stiffness) and functional (reduced vasodilator response) vascular remodeling, which is likely to contribute despite having lower blood pressure. Thus, high circulating levels of aldosterone may increase stroke risk in a blood pressure-independent manner through effects on the cerebral artery wall involving oxidative stress and endothelial dysfunction. NADPH oxidases are potential sources of vascular oxidative stress. This study examined whether aldosterone causes cerebral vascular oxidative stress and endothelial dysfunction, and the involvement of Nox2-containing NADPH oxidase. Control and Nox2-deficient (Nox2−/−) mice were treated with vehicle or aldosterone (0.28 mg/kg/day for 2 weeks) using osmotic minipumps. Aldosterone treatment increased kidney weight, and had no effect on blood pressure. In control mice, aldosterone reduced vasodilation of isolated, pressurised (60 mm Hg) basilar arteries to acetylcholine (ACH; endothelium-dependent agonist) compared to vehicle, suggesting that aldosterone causes endothelial dysfunction. Aldosterone had no effect on ACh responses in Nox2−/− mice. Supplemental 2-hydroxylated metabolites induced cerebral vasodilation, which was blunted in Nox2−/−. Basal and Nox2-stimulated superoxide levels were greater in cerebral arteries of aldosterone vs vehicle-treated mice, and these effects were abolished in Nox2-deficient mice. These data suggest that aldosterone causes cerebral vascular oxidative stress and endothelial dysfunction, and the mechanism involves Nox2-containing NADPH oxidase.

ROLE OF LEPTIN AND INSULIN IN ACTIVATING REINAL SYMPATHETIC NERVE IN HIGH FAT FED RABBITS

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1Baker ID Heart & Diabetes Institute, 2Monash University. In the present study, we determined the rapidity of changes in blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RSNA) in response to a high fat diet using conscious rabbits and whether this involved insulin and leptin actions in the CNS. Furthermore, we examined whether this involved changes to central pathways regulation the response to stress, hypoxia and baroreflexes. New Zealand White rabbits were implanted with telemetry devices to measure BP and RSNA. Rabbits were then placed on a normal or 13.5% high fat diet (HFD) for 3 weeks. Reflexes and stress responses were examined weekly. A separate cohort of fat fed rabbits was infused with peptide antagonists of Leptin (100μg) and Insulin (0.5IU) intra cerebroventricularly at week 1 and 3. After 1 week on the HFD, rabbits demonstrated a 4%, 8% and 30% greater BP, HR and RSNA, respectively (P < 0.05). By the end of 3 weeks of HFD, control and RSNA were elevated by 10%, 5% and 82% (P < 0.05). Baroreflex curve analysis showed that the increase was independent of baroreceptor inputs and similar to the effects of acute stress. Arjet stress induced lesser sympathetic response in fat fed rabbits possibly because this CNS pathway was already activated. The sympathetic-excitatory responses to hypoxia were similar in the HFD and controls over the 3 week period of diet. The insulin antagonist was most effective at 1 week in lowering BP, HR and RSNA while the leptin antagonist was most effective at 3 week of a HFD. The antagonists were minimally effective in control rabbits at these times. Sympathetic activation occurs rapidly within several days of a high fat diet and is associated with forebrain insulin signaling, and later as fat is deposited, with forebrain leptin signaling. These changes appear to be associated with pathways mediating the sympathetic responses to acute emotional stress that are independent of baroreflex or chemoreflex pathways.

PRO-ANGIOGENIC AND ANTI-FIBROTIC ACTIONS OF PROSTACYCLIN EXERTED VIA MODULATION OF NAPDHE OXIDASE 4 IN ENDOTHELIUM AND FIBROBLASTS

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Redox signaling derived from NADPH oxidase (Nox) has important functions in vascular physiology and pathophysiology. The most abundant isoform of NADPH oxidase in vascular cells is Nox4. NADPH oxidase signaling functions are highly dependent on sub-cellular localization and cell type. Up-regulation of Nox4 is known to induce angiogenesis in endothelial cells, whereas in fibroblasts it increases collagen production and produces fibrosis. Prostacyclin (PGI2) is long known to be cytoprotective, but how this signaling intersects with NADPH oxidase in angiogenesis is not known. Transforming Growth Factor-β (TGF-β) induces Nox4 expression both in endothelium and fibroblasts. In endothelium, activation of Nox4 drives cell cycle progression, migration, proliferation, fibrosis, and oxidative stress, and cell proliferation and collagen production. These effects in both cell types were blocked by either adenosine-mediated Nox4 RNAi (Ad-NOX4) or dominant-negative Nox4 (Ad-DNNOX4). TGF-β induced in polynuclear alcohol sponges implanted subcutaneously significantly (n = 6; P < 0.01) increases angiogenesis, indicated by percentage vascular volume (CD31 counts and collagen content (fibrosis) in C5BL/6 mice. These responses to TGF-β were substantially reduced (n = 6; P < 0.01) in sponges instilled with Ad-DNNOX4, suggesting that TGF-β non-selectively induces Nox4 expression in these cells and promote angiogenesis and fibrosis. Interestingly we found that fibroblasts that the infection activates consistently induce Nox4 expression via cAMP/Protein Kinase-A/cAMP-responsive element binding protein pathway. Cicaprost-induced Nox4 in these cells protects them from apoptosis, increases their proliferation, migration and tube formation, and these effects were reduced by Ad-NOX4. In contrast, in fibroblasts, cicaprost suppresses Nox4 expression and reduces TGF-β-induced collagen production and cell proliferation, to a similar extent as Ad-NOX4. Thus prostacyclin signaling selectively up-regulates Nox4 in endothelium and improves angiogenic function. In contrast, prostacyclin down-regulates Nox4 expression and collagen production in fibroblasts and thus reduces fibrosis. Clearly, selective activation of Nox4 via prostacyclin promotes angiogenesis and may limit fibrosis, highlighting how these redox-signaling pathways may be useful for promoting angiogenesis to protect and assist repair of the heart after myocardial infarction.
INHIBITION OF CERAMIDES: A NEW ANTI-OXIDANT THERAPY FOR VASCULAR INFLAMMATION?

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1DAR, Hôpital Bicêtre, Université Paris XI, France. 2Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia. 3LEM, Université Paris VII, Paris, France. 4Réanimation pédiatrique, Hôpital Necker-Enfants Malades, Université Paris V. France. Endothelial inflammation and leucocyte/endothelial interactions are common denominators in many vascular pathologies. The goal of this study was to evaluate the effect of IL-10 on the oxidative stress and endothelial inflammation induced by TNF-α in endothelial cells, and to define the cellular pathways involved. The production of reactive oxygen species (ROS) in human umbilical vein endothelial cells (HUVECs) was studied by flow cytometry using dichlorofluoresceindiacetate (H2DCF-DA). TNF-α (1ng/ml) was added to the perfusion medium, as was IL-10 (10 ng/ml) 30 minutes before TNF-α. PI-3 kinase involvement in the IL-10 inhibitory pathway was assessed using Wortmannin and LY 2940002. The levels of ceramide, and expression of ICAM-1, were measured in HUVECs. We studied the effect of IL-10 on leucocyte/endothelium interaction induced by TNF-α using an ex vivo perfused vessel model. IL-10 significantly reduced H2DCF-DA fluorescence induced by TNF-α in HUVECs (12.5% [-/-] 3.23 vs 111.7% +/- 21.63 at 60 min). Pre-treatment by LY294002 or Wortmannin restored ROS production (TNF-α IL-10 vs TNF-α+IL-10) vs (IL-10 + Wort). 12.5% +/- 2.3, TNF-α IL-10 vs TNF-α+IL-10 + Wort 86.78% +/- 16.38; TNF-α IL-10 vs TNF-α+Wort+TNF-α IL-10 4.44 +/- 1.1 vs 50.21 +/- 12.41 at 60 min). Pre-treatment with-IL-10 in significantly decreased the levels of ceramides in HUVECs (TNF-α vs TNF-α+IL-10) vs (IL-10 + Wort). 6278 +/- 1033 vs 1440 +/- 120.1 pmol/mg prot. IL-10 significantly decreased ICAM-1 expression and leucocyte adhesion (TNF-α+IL-10) vs (IL-10 + Wort). 26.80 +/- 2.66 /% 1.38 adherent leucocytes/field at 15 min). We report the anti-oxidant effect of IL-10 decreases the level of inflammation induced by TNF-α in endothelial cells. This decrease is mediated through PI-3 kinase, and is paralleled by a decrease in ceramide synthesis induced by TNF-α.

DETERMINANTS OF BLOOD PRESSURE IN LATE ADOLESCENCE: GENDER DIFFERENCES AND THE EFFECTS OF BMI AND LIFESTYLE FACTORS

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Background: Lifestyle behaviours established during adolescence may affect blood pressure (BP) and contribute to cardiovascular risk in adulthood.

Aim: To assess the impact of health behaviour related factors on BP in 1248 adolescents aged 17 years in the Western Australian Pregnancy (Raine) Study.

Methods: Associations between BP, and anthropometric, lifestyle and biochemical factors were assessed statistically using linear regression analyses.

Results: Boys had 9 mm Hg higher systolic BP compared with girls (P = 0.001). Girls using oral contraceptives had 3.27 and 1.74 mm Hg higher systolic and diastolic BP, (P = 0.050 and P = 0.004, respectively), compared with non users. Increasing BMI, drinking alcohol in boys, and a higher sodium-to-potassium ratio associated with significantly higher systolic BP, whereas higher levels of self-reported physical activity associated with significantly lower diastolic BP. There was a continuous relationship between BMI and systolic BP in both genders, however this slope of relationship was steeper in boys than girls not taking oral contraceptives (P = 0.028) (Figure 1). Systolic BP in those in the upper quartile of BMI and the urinary sodium-to-potassium ratio, and drinking alcohol (boys) or taking oral contraceptives (girls), was 5.7 and 5.5 mm Hg, respectively, higher than those in the lowest quartile and not drinking alcohol (boys) or taking oral contraceptives (girls).

Conclusion: BP and future cardiovascular risk could be reduced by modifying a range of lifestyle factors in adolescents with appropriate attention to gender-related behaviours.

AORTIC STIFFNESS IS DEPENDENT ON DIRECTION OF MEAN ARTERIAL PRESSURE CHANGE

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It is known that aortic stiffness, as measured by pulse wave velocity (PWV), is dependent on mean arterial pressure (MAP). It has been anecdotally observed that this relationship demonstrates a directional dependence; i.e., PWV is higher at the same MAP when pressure is rising than when it is falling. The exact mechanisms responsible for this are not fully understood. The aim of this study was to quantify this directional dependence in a normotensive and hypertensive strain of rat. Three, 16 week old Spontaneously Hypertensive Rats (SHR) and four, 16 week old Wistar Kyoto rats (WKY) were anaesthetized and two pressure sensors were introduced into the thoracic and abdominal aorta. Pressure was increased with phrenyephrine administered intravascular via a bolus injection or an infusion (30 μg/kg/ min) and allowed to return to baseline. Pressure was decreased in the same manner with sodium nitroprusside. Aortic PW and thoracic MAP were recorded over the entire pressure range. A maximal directional dependence was observed at MAPs of 70mm Hg and 120mm Hg. The directional dependence was greater in the SHR compared to the WKY rats. It was also greater at higher pressure ranges than at lower pressures. Directional dependence was also more pronounced when pressure-altering drugs were administered in a bolus as opposed to a controlled infusion, indicating that there may be a rate-dependent component; that is, the effect is diminished when the MAP changing stimulus is applied slowly. There is likely an interaction between the mechanical and viscoelastic properties of the arteries which determine the direction of directional dependence observed in the PWV-MAP relationship. This information may be useful in understanding altered vascular responses in hypertension and cardiovascular disease.

RELATIONSHIP BETWEEN CENTRAL PULSATILE AORTIC PRESSURE AND MIDDLE CEREBRAL ARTERY FLOW WAVEFORMS IN NORMAL YOUNG ADULTS

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There is inconsistency in the interpretation of the relationship between pulsatile pressure and flow entering the brain in terms of arterial “Windkessel” compliance and resistive properties, and usually done in the time domain only. Interpretations are at variance with those applied to other vascular beds including the kidney, which also has very high blood flow and low resistance. This subject is important if one is to best treat such disease conditions as stroke, cerebral trauma, and neoplasm, and delay progression of the arterial disease which with aging and hypertension causes dementia. In 9 normal young subjects age 28±3 years, radial artery pressure was recorded non-invasively by applanation tonometry and middle cerebral artery flow (MCDF) with transcranial Doppler under control conditions and physiological challenge (Valsalva). Aortic pressure (AP) waves were generated from radial using Sphygmocor®. Comparisons were made of the flow and pressure waves in the time domain as flow (FAx) and pressure augmentation index (PAx), and in the frequency domain as vascular impedance. Mean MCDF was relatively high (62±14 cm/s) and AP 60±6 mm Hg, but within normal limits. PAx was 84 %, and PAx +11 %, also within normal limits for age, but with PAx lower and FAx higher than in older subjects with suspected and confirmed cerebrovascular disease. Impedance modulus was 1.8±10 dynes cm -2 at zero Hz and fell to an average of 0.3±10 dynes cm -2 at 5 Hz, and phase was negative for all harmonics. This is consistent with a capacitive load but dominated by a low resistance vascular bed downstream. Patterns of a low resistive vascular bed persisted during Valsalva manoeuvre. Contrary to previous reports, pressure and flow waves in arteries supplying the brain, both in time and frequency domain, are similar to those in the rest of the body. There is understanding of the mechanical and compliance relatively low. Properties appear to change with age and disease; measurement is practical and may assist in management of hypertensive patients with potential or actual cerebrovascular disease.

PRINCIPAL RESULTS OF THE ANKLE BRACHIAL INDEX DETERMINATION BY OSCILLOMETRIC METHOD IN GENERAL PRACTICE STUDY

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1Menzies Research Institute Tasmania, University of Tasmania. 2Rehabilitation General Hospital, Daw Park, 3Department of Epidemiology and Preventive Medicine, Monash University. One of the simplest and most useful parameters to objectively assess lower extremity arterial perfusion is the Ankle-Brachial Index (ABI). This is rarely done in primary care due to the need for specialised equipment and training. Nearly, 20,000 oscillometric devices were distributed by the High Blood Pressure Research Council of Australia to physicians, mostly general practitioners. The current study, Ankle Brachial Index Determination by oscillometric method IN General practice (ABIDING), sought to expand the utility afforded by these machines in primary care. We sought to determine the agreement between Ankle Brachial index (ABI) measured by Doppler and mercury sphygmomanometer by research nurse and ABI by oscillometric device (OMRON HEM 907) by practice nurse in primary care. We also sought to ascertain the utility of oscillometric devices for the diagnosis of peripheral arterial disease (PAD) in primary care. A cross-sectional validation and diagnostic accuracy study in metropolitan and rural Victorian general practice, conducted between October 2009 and November 2010 of 298 general practices and 14584 patients with cardiovascular disease (CVD) or at high risk (3 or more CVD risk factors) of said. We identified symptomatic individuals by use of the Edinburgh Claudication Questionnaire. Multivariable
INITIAL ORTHOSTATIC HYPOTENSION—THE IMPORTANCE OF A RISE OR FALL IN CARDIAC OUTPUT?
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Active standing is normally associated with a transient reduction in systolic blood pressure (SBP) within the first 20 sec. This can be associated with symptoms of pre-syncopae and occasionally loss of consciousness. The initial hemodynamic response to active standing is complex and a transient reduction in total peripheral resistance (TPR) is a characteristic feature, dependent on cardiovascular autonomic reflexes related to redistribution of blood and skeletal muscle contraction. We studied hemodynamic responses to active standing in 309 medical students of median age 22 (Interquartile range IQR: 2) y, of whom 54% were female. Their median weight was 66.5 (18) kg and height was 170 (14) cm. We used the Finometer Midi device to obtain real-time, beat-to-beat measures of SBP and heart rate (HR) and estimates of cardiac output (CO), stroke volume (SV) and TPR. All subjects rested supine for 5 minutes and values during the last 30 seconds before standing were averaged to provide baseline readings. At baseline median (IQR) values were SBP: 113 (20) mm Hg, CO: 6.1 (2.1) L/min, HR: 70 (16) min⁻¹, SV: 88 (30) mL and TPR: 970 (357) units. Tracings were analysed for changes in the 20 seconds after standing. The maximum transient deviations (Δ) from baseline were recorded for SBP, CO, SV, HR and TPR. These did not necessarily coincide precisely in time and CO sometimes described a biphasic response, in which case, the average of the 2 deviations was used. The median changes were ΔSBP: -44 (23) mm Hg, ΔCO: -0.23 (2.2) L/min, ΔHR: -34 (12) min⁻¹, ΔSV: -34 (24) mL and ΔTPR: -382 (310) units. Qualitatively, there were both rises (n=175, ΔCO: +1.3 L/min) and falls (n=134, ΔCO: -0.8 L/min) in CO. When subjects were grouped as such, there were no significant differences in age, sex, weight or height. However, there were significant differences in the initial hemodynamic responses to standing. Those in whom CO rose on standing had a lesser fall in SBP (–36 v –55 mm Hg, P<0.0001), demonstrated a greater drop in TPR (–454 v –253 units, P<0.0001), and the rise in CO was associated with a slightly greater increase in HR (+34 v +31 min⁻¹, P=0.007) and a lesser decline in SV (–37 v –47 mL, P<0.0001) than those in whom CO fell. Subjects who showed an early fall in CO on standing, those who show an early increase in CO have a slightly higher supine SBP (115 v 111 mm Hg, P<0.0001), dependent on a higher TPR (1051 v 894 units, P<0.0001). They better limit the level to which SBP falls when they stand (79 v 54 mm Hg, P<0.0003), compared with people who show an early increase in CO on standing. These responses might define phenotypic subgroups useful for understanding inter-individual variation in cardiovascular reflexes and the risks of collapse on standing.

A HYPERTENSIVE RESPONSE TO EXERCISE PREDICTS CARDIOVASCULAR EVENTS AND MORTALITY IN APPARENTLY HEALTHY INDIVIDUALS: A SYSTEMATIC REVIEW AND META-ANALYSIS
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Exercise stress testing is routinely undertaken throughout the world. Studies have shown that a hypertensive response to exercise increases cardiovascular (CV) risk. We conducted systematic review and meta-analysis to determine the predictive value of exercise BP for CV events and mortality. We reviewed nine longitudinal studies in a total of 48,262 apparently healthy individuals free of coronary heart disease. Total CV event and mortality rates were recorded over a mean follow-up of 16 years. Independent of resting BP, age and CV risk factors, there was a trend for exaggerated exercise systolic BP (>180 mm Hg) at moderate workloads to be associated with increased rate of CV events and mortality (Adjusted hazard ratio; HR range 0.97–2.37, 95% CI range 0.68–4.69). Exaggerated exercise systolic BP at maximal workloads also increased the rate of CV events and mortality (Adjusted HR range 1.13–2.47, 95% CI range 0.71–4.18). A meta-analysis was conducted on data from four studies that provided HR for exercise systolic BP on a continuous scale, and this included 12,648 participants followed over a mean of 17 years. After adjustment for age, resting BP and CV risk factors, there was an 8% increase in rate of CV events and mortality per 10 mm Hg increase in exercise systolic BP at moderate intensity (P=0.05; Figure 1). A hypertensive response to exercise is an independent risk factor for CV events and mortality in healthy individuals, highlighting the need to determine mechanisms and appropriate management of patients with exercise hypertention.

HOW RELIABLE IS VISIT-TO-VISIT CLINICAL BLOOD PRESSURE VARIABILITY?
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Approximately 2.1 million Australians have hypertension (ABS National Health Survey 2004–05). Increased visit-to-visit variability in systolic blood pressure (SBP) is a significant predictor for all-cause mortality, although instability in clinic BP measurements may be considered less sensitive and specific than home or ambulatory measurements. Our aim was to measure within-person variability in clinic BP compared to 24 hour ambulatory BP in the same subjects and included subjects referred to our Service during 2010. During two consecutive clinic visits, we measured three resting seated BP measurements, 5 minutes apart using a mercury sphygmomanometer by two trained non-medically qualified professional staff. The mean of the second and third measurements from each visit was compared using paired t-tests. We then compared these clinic measurements with mean 24 hour awake BP from the ambulatory study. We had 117 participants (49M:68F) with age ranging from 18–88 years [mean 60 ± 0.73 (SD) years]. Mean BP (±SD) did not significantly vary between the 2 clinic visits (144 ± 20/80 ± 11 vs 145 ± 18/80 ± 10 mm Hg) and was similar to 24-hour mean awake BP (134 ± 13/79 ± 8 mm Hg). Pearson’s correlation of mean clinic SBP with mean 24 hour awake SBP confirmed this agreement (Fig. 1, r=0.73, P<0.005). It is therefore important that BP variability for the individual patient is identified to tailor treatment.

REGULAR CONSUMPTION OF A WILD GREEN OAT EXTRACT ENHANCES SYSTEMIC AND CEREBRAL VASODILATOR FUNCTION
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Impaired endothelial dilatation in systemic and cerebral arteries is associated with future cardiovascular and cerebrovascular events. Various bioactive nutrients have been shown to have acute vasodilator effects but only a few appear efficacious following chronic consumption. Green oat extracts contain various bioactive ingredients includingavenanthramides, which may improve endothelial function. A randomised, double-blind, placebo-controlled crossover trial of 24 weeks’ duration was conducted in 38 healthy adults aged 67 ± 8 years to see whether dietary supplementation with 1500 mg/day of a wild green oat extract (Neuravena®; Futteram Switzerland) would elicit sustained changes of vasodilator function. Flow-mediated dilatation of the brachial artery (FMD) was assessed at the end of each 12 week treatment period in 33 adults, after overnight fasting and at least 24 hours after taking their last dose of supplement. In 18 adults cerebral vasodilator response (endothelium-dependent) was also assessed using transcranial Doppler ultrasound to measure change of blood flow velocity in the middle cerebral artery during inhalation of Carbogen gas. We observed treatment-induced increases of similar
Antithrombotic therapy is beneficial for prevention of cardiovascular disease, but is also associated with increased risks of bleeding. Although a number of observational studies demonstrated strong associations between blood pressure (BP) and antithrombotic therapy-related intracranial bleeding, there remains uncertainty about the effects of BP lowering treatment on the risks of bleeding associated with antithrombotic therapy. As a result many guidelines for prevention of cardiovascular disease do not refer to the importance of BP control during antithrombotic therapy. The objective of the present analysis is to determine whether BP lowering provides protection against major intracranial and extracranial bleeding events associated with antithrombotic therapy. This is a subsidiary analysis of the Perindopril Protection Against Recurrent Stroke Study (PROGRESS) - a randomized, placebo-controlled trial that established the beneficial effects of BP lowering in secondary prevention of stroke. A total of 4876 patients with antithrombotic therapy at baseline were assigned to either active treatment (perindopril in all participants plus indapamide for those with an indication for, or contraindication to, a diuretic or matched placebo) or placebo. The outcomes were major intracranial bleeding (intracerebral haemorrhage, subarachnoid haemorrhage and subdural haematoma) and extracranial bleeding. Over a mean follow-up of 3.9 years, 66 intracranial and 108 extracranial bleeding events were observed. Active treatment lowered BP by 2.0/1.3 mm Hg and reduced the risk of intracranial bleeding by 46% (95% CI 38 to 55%). However, active treatment did not reduce the risks of extracranial bleeding (mainly gastrointestinal bleeding; relative risk reduction – 2% [95% CI –50 to 30%]). The analyses of achieved follow-up BP showed that the lowest risk of intracranial bleeding was observed among participants who achieved the lowest follow-up systolic BP levels (median 113 mm Hg). In contrast, there were no clear associations between achieved follow-up BP levels and the risks of extracranial bleeding. In conclusion, BP lowering is likely to provide protection against intracranial bleeding associated with antithrombotic therapy.
represent an ideal unlimited source of autologous CMs. hPSC treated with trichostatin-A (TSA) (1 ng/mL) during embryoid body formation yielded beating CMs that expressed several key cardiac markers, cyclized calcium and were responsive to chronotropic agents (isoprenaline and carbachol).

However, implanting undifferentiated or a mixed population of hPSCs in the tissue engineering chambers resulted in teratoma formation at 4 weeks. Therefore, hPSC-derived CMs need to be expanded to minimize the risk of teratoma formation. In a proof-of-concept study, we used a fluorescent mitochondrial dye, tetramethylrhodamine methyl ester perichlorate (TMRRM), to sort cells by FACs, resulting in two populations of neonatal rat CMs. The TMRRM-high cells consisted of troponin-I positive CMs that maintained spontaneous contractions for 6 months while TMRRM-low cells were non-contractile and troponin-I negative. Therefore, TMRRM staining and sorting suggests a viable method to enrich hPSC-derived CMs. In conclusion, we have presented multiple strategies (cytosepation with IPC, cardiac differentiation with small molecule TSA, and CM enrichment with TMRRM) that advance us towards generating transplantable human cardiac constructs.

DIFFERENTIAL REGULATION OF TP ISOFORMS IN PREECLAMPSIA

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Preeclampsia (PE) is a serious complication of pregnancy unique to humans. In Australia, approximately 10% of all pregnancies are affected by either PE or Intrauterine growth restriction (IUGR), making these diseases a serious health risk to both mother and baby, and a significant health care burden. PE describes the syndrome that precedes the disease eclampsia and primarily includes onset of (1) hypertension and (2) proteinuria after the 20th week of gestation. Pregnancies affected by PE and IUGR are characterised by poor placental development and a lack of vascular remodelling. Spontaneous PE is unique to human pregnancy and does not occur in other species without intervention to precipitate the condition. Recent evidence suggests that isoprostanes, ligands for the TP receptor, are strongly linked to the pathogenesis of PE. Our hypothesis is that TPβ promotes PE through perturbing normal placentalisation or causing vascular dysfunction.

Immunohistochemistry of placental biopsies indicates that the human-specific TP isoform is up-regulated in the syncytiotrophoblast layer of the placenta in PE and IUGR. TPβ expression does not alter between normal pregnancy and PE. Like normal placenta, BeWo cells (a human placenta syncytiotrophoblast cell line) express only TPα. Overexpression of TPβ in BeWo cells inhibits proliferation, induces cell cycle arrest and prevents forskolin-induced syncytialization. BeWo cells transfected with TPα synchronize at a much faster rate than control cells. These data suggest that TPα is required for normal placentation whilst TPβ expression produces pathological abnormalities in placentalization that could contribute to the development of conditions such as PE. The unique C-terminal residues of TPβ are the likely source of the signalling that perturbs placenta. In support of this hypothesis the placenta of the TPβ−/− mouse is ischemic and underdeveloped showing many of the hallmarks of IUGR/PE. These data suggest that the human-specific isoform TPβ may regulate the onset and severity of PE through manipulation of trophoblast function in addition to its suppression of angiogenesis.

β-AMINO ACID SUBSTITUTION OF ANGIOTENSIN III IMPARTS SIGNIFICANT AT2 RECEPTOR SELECTIVITY AND FUNCTIONAL VARIABILITY

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The existence of multiple angiotensin II (Ang II) receptor subtypes has been recognised for many years yet there still remains a frustrating lack of selective pharmacological tools with which to probe Ang II type 2 receptor (AT2R) function. Using native Ang II as a template, we have recently shown that a single β-amino acid substitution in Ang II altered the specificity and proteolytic stability, which translated into AT2R-mediated vasoconstriction both in vitro and in vivo. Since native Ang III has greater binding affinity than Ang II at AT2R, we reasoned that a similar approach of β-amino acid substitution, but using Ang III as a template, would yield peptides with even greater AT2R selectivity. A group of peptides was synthesised by sequential β-amino acid substitution of the naturally occurring α-amino acids throughout the Ang III peptide sequence with the corresponding β-amino acid. Binding experiments were conducted in AT1R- and AT2R-transfected HEK-293 cells to determine relative AT1R- and AT2R-selectivity of these novel β-peptides. The rank order of relative affinity at AT1R and AT2R found to be β-Pro > β-Tyr > β-Phe > β-AngI > β-Val > β-Leu = Ang II. Notably, β-Pro Ang III showed > 40,000-fold selectivity for AT2R compared to AT1R. Moreover, β-Ang III peptides demonstrated varying degrees of AT2R-mediated vasoconstriction in mouse isolated aorta, the magnitude of which showed close correlation with AT2R binding affinity. Indeed, β-Pro Ang III caused ~ 30% reversal of pre-contracted vessels that occurred via classic AT2R-mediated signalling pathways, thus demonstrating similar binding and functional characteristics to that of the only commercially available AT2R agonist, CGP42112. Thus the current approach to design and synthesis novel AT2R agonists and antagonists should consider selective ligands to provide insight into AT2R function, and may lead to the development of future AT2R-selective therapeutics in the fight against cardiovascular pathologies.
stimuli (the open field, elevated plus-maze and social interaction tests), behavioural measures of anxiety and cardiovascular arousal were similar between genotypes. However, after-test recovery of blood pressure was faster in AT 1A−/− mice. Conversely, in response to a panicogenic stimulus, footshock, AT 1A+/+ mice showed decreased flight (−24%, p < 0.01) andpressor reactions (−32%, p < 0.002). Likewise, AT 1A−/− mice displayed reduced flight behaviour (escape from the open arm) in the elevated T-maze task (−24%, p < 0.02). During re-exposure to the context (footshock chamber), AT 1A+/+ mice also showed decreased freezing andpressor responses. In AT 1A−/− mice, the re-exposure-induced c-fos expression was markedly (−10 fold) attenuated in the peri-aqueductal grey, a midbrain area that is critical for the expression of stress-flight responses. Baseline c-fos expression in the hypothalamic nuclei was similar between genotypes. These data suggest that AT 1A receptor knockout decreases cardiovascular and behavioural reactions to panicogenic, but not anxiogenic stimuli in mice. AT 1 receptors may thus be a potential target to selectively control pressor and behavioural components of panic-like reactions, without compromising general cardiovascular reactivity and risk assessment behaviour.

ENDOTHELIN-1 VASCULAR ACTIVITY IN HUMAN AND RAT REGIONAL BEDS: A ROLE IN PULMONARY HYPERTENSION?

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Endothelin-1 (ET-1) is a potent vasoconstrictor peptide suggested to play a major role in the pathogenesis of pulmonary hypertension, a disease characterised by pulmonary arterial remodeling leading to right heart failure. Endothelin antagonists are currently first line treatment for pulmonary hypertension, however they are unable to prevent disease progression. Recently, it has been proposed that calcitonin gene-related peptide (CGRP) may act to balance the contractile effects of ET-1 in vivo. The aims of this study were to investigate the efficacy of bosentan, in attenuating ET-1 induced contractions in pulmonary arteries and systemic arteries to determine if CGRP was able to reverse these contractions. The effects of bosentan, a non-selective ET A/ET B receptor antagonist, on endothelin-1 induced contractions were investigated in isolated pulmonary, mesenteric and tail arteries from rats. Relaxation to CGRP was measured in ET-1 pre-contracted arteries. In addition, CGRP was applied in ET-1 pre-contracted isolated human radial and pulmonary arteries collected from coronary bypass and lung resection surgery. ET-1 (0.1–300 nM) caused concentration-dependent rightward shifts of the ET-1 concentration-response curve in rat mesenteric and tail (P = 0.05 vs. vehicle control), but was unable to relax either rat or human pulmonary arteries. These data suggest that bosentan is effective in attenuating the effects of ET-1 in systemic, but not pulmonary arteries. CGRP was unable to relax pulmonary arteries, suggesting it may not play a role in regulation of vascular tone in this circulation.

THE EFFECT OF CHRONIC TREATMENT WITH DIAZEPAM ON STRESS AND HYPERTENSION IN SCHLAGER BPH/2J HYPERTENSIVE MICE

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Schlagher hypertensive mice (BPH/2J) are thought to have neurogenic hypertension. They also show a greater BP increase with the onset of the dark when they become active. This induces greater activation in BPH/2J mice in the daytime inactive period compared with normotensive (BPN/3J) mice. Additionally the MeAm is important to the hypertension in the throracic aorta (TA) has the capacity to accommodate high pulsatile pressure during cardiac systole whereas the abdominal aorta (AA) is less distensible. This regional variation may be altered in conditions when stiffening occurs, such as aging, hypertension and kidney disease. Further complicating blood flow regulation is the risk of end organ damage. The aim of this study was to determine regional variation of aortic biomechanical properties in a rat model of chronic kidney disease with associated hypertension, comparing the Lewis Polycystic Kidney rat (LPK) to Lewis controls. Unbranched, 2–4 mm long distending TA rings and AA rings (distal to the renal bifurcations) were obtained from animals aged 12–18 weeks. Aortic rings were assessed using a uniaxial tensile test in which the aortic segments were stretched at 2 mm/min until breaking point. Results were determined by calculating stress and strain at break, thereby providing measures of the elastin modulus (EM), collagen modulus (CM) and the area under the stress-strain curve which represents the energy absorbed (EA) by the sample. Strain at break did not differ between TA and AA of LPKs but did differ in Lewis controls (Δs ± 2.5 ± 5.5 %, P < 0.02), leading to a lower magnitude of change in strain between TA and AA in LPK relative to Lewis (−8 ± 3 vs 11 ± 4 %, P = 0.005). A greater stress at break was observed for AA compared to TA for both groups (P < 0.0001) but the magnitude did not differ between the two strains. AA had higher CM compared to TA in LPK (0.8 ± 0.1 vs 0.4 ± 0.02 MPa, P < 0.0001), resulting in a greater change in EM between TA and AA in LPK compared to Lewis (0.4 ± 0.1 vs 0.1 ± 0.1 MPa, P = 0.007). CM and EA values for AA were higher than TA in both experimental groups but there was no significant difference in measures between the stress-strain results suggesting that LPKs have a higher gradient of stiffness along the descending aorta, which may alter the characteristics of flow in the vessel and contribute to the increased pulse pressures and hypertension seen in these animals.

IS THERE DIFFERENTIAL PROGRAMMING BETWEEN THE SEXES OF ADULT RENAL FUNCTION AS A RESULT OF EARLY LIFE GROWTH RESTRICTION?

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The aim of this study was to compare renal function in adult male and female rat offspring that had been growth-restricted in early life. Early life growth restriction was induced in Wistar-Kyoto rat offspring by administering a low protein diet (8.7% casein) to the dams during pregnancy and for two weeks during lactation. Offspring of rats fed a normal protein diet (20% casein) were used as controls. At 32 weeks of age, kidney structure and function were comprehensively assessed in male and female offspring using M mode and Doppler ultrasound and 1H and 13C para-aminohippurate clearance techniques. Body weight at 32 weeks was similar across the groups, whereas the attenuation of nephron number, with females exhibiting a greater loss, was evident in the offspring exposed to maternal protein deprivation. Weight relative kidney length was significantly increased in growth-restricted offspring (9% increase in males and 7% increase in females). Conscious mean arterial blood pressure and heart rate were unchanged in growth-restricted offspring. Overall, there was evidence of hyperfiltration in the growth-restricted offspring with GFR/kg weight and filtration fraction altered. Furthermore, proteinuria expressed as % of renal blood flow was increased while glomerular filtration rate expressed as % of renal blood flow increased (15% increase in filtration fraction in IUGR male and 14% increase in IUGR female kidneys) indicative of glomerular hyperfiltration. Overall, the effect of IUGR on renal function was not different between male and female offspring in adulthood. In general, female offspring exhibited a higher level of renal function when compared to male offspring. Although early life growth restriction altered renal function in adulthood, there was no evidence of differential programming of impaired renal function between the sexes.
NADPH OXIDASE NOX2 FACILITATES RETINAL NEOVASCULARISATION IN A MOUSE MODEL OF OXYGEN-INDUCED RETINOPATHY

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It is well established there is a correlation between retinal vasoconstriction and the incidence of coronary heart disease. Both conditions share some clinical features like impaired blood flow and increased vascular inflammation. Abnormal blood vessel growth resulting from retinal ischaemia is a leading cause of vision impairment in proliferative diabetic retinopathy and macular degeneration. Accumulating evidence implicates a reactive oxygen species (ROS)-generating enzyme NADPH oxidase is involved in retinal neovascularisation. Therefore we examined how NADPH oxidase regulates neovascularisation in a mouse model of oxygen-induced retinopathy (OIR). Because Nox2 is a major isoform expressed in endothelial cells and involved in vessel growth, we compared the degree of retinal vascularisation between Nox2-deficient (KO) and wildtype mice in OIR. As shown, retinal vascularisation in OIR was significantly reduced in Nox2 KO mice (P<0.001), indicating that Nox2 facilitated retinal vascularisation in OIR. Decreased vascularisation was accompanied by a reduction in VEGFR1 mRNA expression (by 20%), suggesting an interaction between Nox2 and VEGF was involved in retinal vessel growth in OIR. To confirm our findings, immunohistochemical localisation of Nox2 and VEGF proteins, ROS signals and endothelial cells are currently under investigation. In conclusion, Nox2 is an important signalling mediator involved in retinal vasoconstriction, possibly via a VEGF pathway in OIR. Our findings suggest an additional avenue for intervention to suppress neovascularisation associated with proliferative retinopathy, and perhaps vision loss associated with macular degeneration.

THE INFLUENCE OF GLUTATHIONE PEROXIDASE ON EMOTIONAL AND CARDIOVASCULAR RESPONSES TO ASSURSE STRESS IN MICE

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Free radicals are produced in response to emotional stress. One major anti-oxidant enzyme responsible for eliminating free radicals is glutathione peroxidase. However, the role of glutathione peroxidase in the behavioural and cardiovascular responses to averse stress remains elusive. We examined fear-related behavioural reactions and associated cardiovascular arousal in the glutathione peroxidase 1 knockout (GPX1 KO) mice, using synchronized video and blood pressure telemetry monitoring. In the elevated plus maze test, anxiety-like behaviour and cardiovascular arousal were similar between genotypes. In response to a discrete aversive stimulus, footshock, and subsequent re-exposures to the context (footshock chamber) no differences in cardiovascular reactivity were observed. However GPX1 KO mice displayed approximately twice the immobility when re-exposed to context compared with control animals. In subsequent retention tests, GPX1 KO mice also showed accelerated extinction of freezing. In addition, GPX1 KO mice show higher levels of risk assessment behaviour (stretched-attent posts) in food presentation tests. These data suggest that systemic GPX1 inhibition in mice increases the behavioural response, but not cardiovascular reactivity, to averse stressors. This study indicates that GPX1 may play a role in general emotional reactivity and risk assessment behaviour, but has a limited effect on cardiovascular parameters.

RESEARCH RELATIONSHIP BETWEEN CENTRAL PULSATILE AORTIC AND INTRACRANIAL PRESSURE IN HUMANS

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Logical management of hypertension and its neurological complications requires understanding of the mechanisms determining pulsatile cerebral blood flow, their disturbance in disease, and their amelioration with therapy. To date, emphasis has been directed to compliance of arterial and arteriolar vessels within the skull. This approach differs with approaches addressing pulsatile function in vascular beds elsewhere. In 10 patients with normal pressure hydrocephalus (NPH), the relationships between central aortic pressure (AP) and intracranial pressure (ICP) were compared with a study prior to establishment of a required cerebrospinal fluid shunt to the pleural cavity. Pressures were measured from within the radial artery and cerebral ventricle with matched, fluid-filled, high-frequency manometers. Radial pressure waves were converted to aortic waves using SphygmoCor®. Simultaneously recorded AP and ICP waves were ensemble-averaged and compared in the time and frequency domains. Basic characteristics of patients were: diagnosed NPH with typical clinical features; age 76 ± 4 years, males, systolic 149 ± 19 mm Hg, diastolic 62 ± 13 mm Hg, mean AP 90 ± 8.8 mm Hg, mean ICP 0.5 ± 3.7 mm Hg, pulse AP 60 ± 13 mm Hg, pulse ICP 6 ± 2 mm Hg.

ICP and AP pressure waves were similar in shape and ratio of ICP/ AP amplitude was 0.08 ± 0.01. Similarly in the time domain was confirmed by similarity of ICP/ AP of the first 3 harmonics (which contained 98% of waveform energy) and mean phase delay close to or not significantly different from zero. Close correspondence of ICP and AP pressure waveforms in both time and frequency domains indicates ICP pulsations are due to pulsations of pressure in central arteries without any appreciable effect of venous pressure. Since aortic pressure pulsations are markedly effected by wave reflection from the lower body, one must consider effects of drugs on systemic wave reflection when attempting to reduce fluctuations of ICP.

ACUTE INTERMITTENT HYPOXIA INDUCED LONG-TERM FACILITATION OF SYMPATHETIC NERVE ACTIVITY IS SEROTONIN DEPENDENT

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Acute intermittent hypoxia (AIH) is a commonly studied experimental model of obstructive sleep apnoea (OSA). AIH can elicit a long-term increase in sympathetic outflow (long-term facilitation, LTf), however the underlying mechanism is not clear. In this study, we aimed to investigate the role of serotonin in the development of sympathetic LTf. In urethane-anesthetised, vagotomised and mechanically ventilated Sprague-Dawley rats, we investigated the effect of ten episodes of 10%/90% O2, on sympathetic chemoreceptor and baroreceptor reflex 60 min after AIH. AIH elicited a robust increase in SNA (+56.1 ± 7%; n=10, P<0.001) compared with time controls (rats not exposed to hypoxia, n=5) 60 min after the end of AIH. After the establishment of sympathetic LTf, the hypertensive chemoreflex was enhanced (peak change from 92±22 to 186±38%; baseline vs. after AIH, P<0.05), and the sympathetic baroreflex sensitivity was increased (Gainmax from 7.9±1.0 to 2.60±0.28mmHg-1; n=8, P<0.05). Pre-treatment with systemic methysergide (4mg/kg, i.v., n=8), a broad spectrum serotonin receptor antagonist, attenuated the AIH induced SNA (+20.1±7.6%; P>0.05 compared with time controls). However, the enhancement of hypertensive sympathetic chemoreflex reflex sensitivity was abolished (Gainmax, from 3.16±0.71 to 2.32±0.50mmHg-1; n=6, P<0.05) Our findings indicate that the AIH-induced sympathetic LTf requires 5-HT receptors, however, the enhancement of the hypertensive sympathomotor reflex is likely mediated by non-serotonergic mechanisms.

RENAAL SYMPATHETIC NERVE ABLATION IN MODERATE TO SEVERE CHRONIC RENAL FAILURE: A SHORT TERM SAFETY AND EFFICACY PILOT STUDY

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Sympathetic activation is a cardinal feature of chronic renal failure (CRF) and contributes to poor cardiovascular outcomes. Renal sympathetic nerves play a crucial role in blood pressure (BP) regulation and hypertension commonly associated with CRF. Renal denervation (RDN) has been proven in resistant hypertension with normal renal function. Whether this approach is safe and effective in high risk patients with resistant hypertension and an estimated glomerular filtration rate (eGFR) below 45ml/min/1.73m² has not yet been explored. In this pilot study, embolisation radiofrequency ablation was performed in 4 hypertensive patients (age 64 ± 8 yrs; BMI 33.10 kg/m², mean±SD) with CRF (stage 3-4) (mean baseline eGFR 26.9 ± 9.2 ml/min/1.73m²). To minimize contrast exposure and to reduce the risk of contrast induced nephropathy CO2- angiography was used to visualize the renal arteries and to position the ablation catheter. Standardised office BP readings and serum biochemistry were obtained before and at 1, 3, 6 month follow-up. Mean baseline BP was 188±26/91±23 mm Hg despite an average of 5.0±2.1 antihypertensive drugs. Each patient underwent bilateral denervation in one session with an average of 4.6±1.3 ablation treatments per artery. To assess effect of RDN on arterial stiffness we measured finger tip augmentation index (AI) using peripheral arterial tonometry (EndoPAT 2000) before and 3 month after RDN. Mean eGFR was 24.8 ± 8.8, 24.5 ± 9.7, 27.9 ± 9.9 ml/min/1.73m² at 1, 3, 6 month follow-up. Average office-seated BP decreased by 34/14, 22/14, 17/19 mm Hg for systolic and diastolic BPs at 1, 3, 6 month follow-up. RDN significantly reduced peripheral AI (51.31 vs. 38.33 %, P=0.01), at 3 months follow-up. Angiographic evaluation before and directly after the procedure did not reveal any compromise of the treated arteries. There were no intra- or periprocedural complications. RDN can be performed safely and effectively in high risk patients with CRF stage 3-4 without short-term adverse consequences on renal function. RDN exerts a beneficial effect on BP control and reduces arterial stiffness. These preliminary findings indicate that RDN attenuates the mechanisms underlying hypertension-related cardiovascular morbidity and mortality in CRF.
Abstracts From the 33rd Annual Scientific Meeting of the High Blood Pressure Research Council of Australia

Hyptension, 2012;60:487-502
doi: 10.1161/HYP.0b013e3182602164

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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