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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 wild-type (WT) littermates (n=12-14/group) 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 HWBW 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 vascular and cardiac-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 RECOVERY 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 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 (-0.755, P=0.012). During exercise, there were significant increases in forward components of waves in early systole (baseline 27.02±10.5=15.71±10.0 Wm−2 vs. exercise 41.01±10.6=21.09±10.0 Wm−2, +62 %, P<0.014, corresponding to peak ejection) and forward decompression waves in late systole (baseline 10.96±6.40±6.40±10.0 Wm−2 vs. exercise 20.88±5.79±10.0 Wm−2, +127 % 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.

PRIMARY ENDPOINT RESULTS FROM THE VALSARTAN INTENSIFIED PRIMARY CARE REDUCTION OF BLOOD PRESSURE (VIPER-BP) STUDY: A MULTICENTRE, RANDOMISED TRIAL

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Purpose: Elevated blood pressure (BP) remains poorly controlled in primary care despite effective antihypertensive drugs.

Design: The Valsartan (Intensified Primary care) Reduction of Blood Pressure (VIPER-BP) Study, was a national multicentre randomised trial involving 259 General Practitioners from 114 clinics Australia-wide. The study compared usual primary care (enhanced by automated absolute risk profiling) with an intervention of structured BP reduction and risk management (also comprising automated risk profiling plus standardised pharmaceutical treatment). For computer assisted, intensified follow-up and treatment titration, Following a 14–28 day run-in period (valsartan 80mg/day), hypertensive patients who remained above their individualised BP target were randomized to usual care or the VIPER-BP intervention arm (comprising initial valsartan mono-therapy or 2 forms of valsartan combination therapy-ratio of 1:2). The primary end-point was individualized BP control (NHFA expert guidelines) at 6 month follow-up. Secondary endpoints included change in BP, absolute risk of cardiovascular disease (CVD) and safety profile. The trial design provided > 80% power to detect a minimum 7% absolute difference between groups in BP control at 6 months at a two-sided significance of 0.05.

Study Cohort: A total of 2357 hypertensive patients were enrolled and 2183 (59±12 years, 59% men and 60% prior hypertension) with a mean BP of 154±17/91±11 mm Hg entered the run-in phase. Subsequently, 462 (21%) achieved their individual BP target (126±8/76±8 mm Hg) and 159 (7%) withdrew. Overall, 1562 patients (59±12 years, 62% men and BP of 149±17/76±11 mm Hg) with a DVD-free target of 140/90 mm Hg (20%), pre-existing CVD (53.5%, target 130/80mmHg) or renal impairment (17.5%, target 125/75 mm Hg) remained above their BP target. Patients were randomized to usual care (524, 34%) or the VIPER-BP intervention (1038, 360 and 678 patients assigned initial mono- or combination valsartan therapy, respectively). The groups were well matched according to their demographic and clinical profile (including individual BP targets).

Results: With follow-up completed in September 2011, the primary and key secondary endpoints will be presented at the meeting.

PREDICTING PREGNANCY OUTCOMES: SMALL OR EARLY?

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Being born small or early clearly has important implications for lifelong human 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 different 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 development of organs such as the kidney, heart and skin depending upon sex. This suggests future treatment of a mother with prenatal glucocorticoids should take into account the fetal sex.

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 signalling 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 hypoxemia 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 in fetal and newborn life. Recognizing the need for evidence beyond a-pcap size, we have investigated mechanisms mediating hypoperfusion, and the role vasoconstriction plays in supporting fetus to newborn. Most important are the post-hypoxic responses of the fetus, the after birth as part of post-hypoxic recovery. Thus while the fetus has transitioned to birth weight and 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 signalling pathways in the heart in 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-2R signalling pathway, and inhibitors of protein kinase C. Leu27IGF-2 increased the area of cultured cardiomyocytes, but not mononucleated, cardiomyocytes. Inhibition of PKC with G6976 did not prevent the Leu27IGF-2 induced increase in the cell area of bineucleated 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 renal 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 fetal blood pressure in the face of transient impairment of cardiac output. There are a number of complications faced by the newborn which further impair cardiac function such as a patent ductus arteriosus and the changes in metabolic demand of organs such as the kidney and newborn. There is value in taking a physiological approach to understanding the transition from fetal to newborn life, recognizing the need for evidence based science to develop and refine ways of improving perfusion of the kidneys and other vital organs in premature babies.

CARDOVASCULAR AND METABOLIC OUTCOMES OF OFFSPRING BORN SMALL OR EARLY: THE RAINE STUDY

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Extensive epidemiological evidence confirms that being born small is associated with increased cardiovascular and metabolic risk. Increasingly it is understood that it is suboptimal in-utero environment that is the precursor for fetal programming. The West Australian Pregnancy Cohort (RAINE Study) recruited 2900 pregnant women in 1989—1990. Two thousand and eighty six neonates were born. The offspring have been followed up at birth, 1, 2, 3, 5, 8, 10, 14 and 17 years of age. Blood pressure was measured at each follow up. Fasting blood samples were taken at 8, 14 and 17 years. From this longitudinal prospective study, we have investigated the role of early life factors on increased cardiac-metabolic risk. Specifically we have investigated the role of low birth size, high birth size (Huang et al., 2006), maternal obesity, maternal obstetric complications, childhood growth trajectories (Huang et al., 2011), lifestyle, (O’Sullivan et al., 2010) and the role of epigenetic influences upon these outcomes. The conglomeration of influences upon increased cardiac-metabolic risk (Huang et al., 2009) in this contemporary Australian population cohort, indicate that, in addition to low birthweight, many other suboptimal in-utero and early postnatal conditions increased cardiovascular risk. We have confirmed in this population, that there is a U shaped relationship between birth size and cardiac-metabolic risk (Figure) (Huang et al., 2007).

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

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Introduction: Lifestyle throughout the lifetime course makes an important contribution to development of metabolic and cardiovascular disease. Diet, physical activity and smoking are among the well established lifestyle determinants that affect cardiovascular 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 early life of these born small for gestational age can prevent or reduce 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 technologies 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 on birth weight are small, but reduce the risk of either very low or very high birth weight (Juhti 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 low birth weight and metabolic disease is lost in fit individuals, and consistently, that the association between low birth weight and metabolic syndrome is attenuated in unfit individuals (Laaksonen et al., 2003). Interestingly, genetics and early habit format may, therefore, play a more likely role in the physical activity of the brain mass, beta-cell mass and function, as well 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 mmHg of arterial pressure in the stroke-prone spontaneously hypertensive rat (SHRS). This congenic interval contains a number of candidate genes, including genes involved in aldosterone synthesis. We hypothesised that introgression of this congenic region from the normotensive WKY into the hypertensive SHRS may restore aldosterone regulation and reduce salt-sensitivity in the SHRS. Hemodynamic variables were measured by radiotelemetry from 12–21 weeks of age in WKY, SHRS and our congenic strain (SP.WKY10cM) with the congenic interval (10 cM) from the WKY introgressed into the SHRS 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 cage 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 mmHg to 147 ± 3 mmHg, SP.WKY10cM: 170 ± 7 mm Hg to 201 ± 4 mm Hg and SHRS: 194± 5 mm Hg to 235 ± 6 mm Hg (P < 0.001). SHRS 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 adrenal 3βHSD and 3βHSD mRNA expression compared to WKY, genes located in the chromosome 2 congenic interval involved in the aldosterone synthesis pathway. There was no difference in excretion parameters or adrenal mRNA gene expression between SP.WKY10cM 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 ONrenal 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 fortnightly 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 controls (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 INFLTRATION 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 AT1 receptor (AT1R), a direct AT1R stimulation has been shown to affect inflammatory actions, the study investigated whether an AT1R agonist, CGP42112, could prevent T cell activation and vascular infiltration 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 (AT1R antagonist; 1 mg/kg/day), Ang II + CGP42112 (114 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 systolic blood pressure (Ang II: 154±4 mm Hg 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 effecter T cell marker, CD44+ (Vehicle: 12.4±0.9 % Vs Ang II 20.6±1.4 %, P<0.001; 1-way ANOVA), was significantly elevated compared to vehicle-treated mice. Interestingly, CGP42112 reversed Ang II induced CD4+ T-cell activation to a greater extent than AT1R blockade, but it also increased cytokotoxic T cell (CD8+) infiltration into the aorta and kidney was significantly attenuated in mice treated with CGP42112 or candesartan cilexil. Collectively, our findings demonstrate AT1R-evoked suppression of the adaptive immune system in hypertension, and may represent a novel strategy for the treatment of inflammation associated with cardiovascular disease.

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. QOL scores at baseline were compared to 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 adenectomy. 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 for 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 QOL 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.

HIGH INTRALUMINAL PRESSURE INDUCES VASCULAR INFLAMMATION

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Hypertension is a potent contributing factor to atherosclerosis and coronary artery disease but the exact mechanism by which the former causes the latter remains elusive. While previous reports suggest that such factors as the rennin-angiotension system are responsible, we explore whether high intraluminal pressure per se induces atherosclerosis and hypothesise that this may be due to the induction of inflammation and the adhesion cascade. We previously reported that high intraluminal pressure increases leukocyte adhesion to the endothelium in harvested vessels. We now show that high intraluminal pressure increases expression of adhesion molecules in arteries and the number of endothelial microparticles from HUVECs under flow pressure (control: 159±16 vs 120 mm Hg; 317±37; n=3; P<0.05). Changes in the mechanical forces exerted on the endothelium were studied. Vessels placed under pressure but at static flow demonstrated an increase in leukocyte adhesion. Low shear stress (LSS; 1.6 dynes/cm2) coupled with pressure (120 mm Hg) induced increases in leukocyte adhesion compared to HSS (16 dynes/cm2; 90±23 vs 24±9 leukocytes/field respectively; n=5–6; P<0.001). However, LSS on its own (with no pressure) had no influence on leukocyte adhesion (4±3 leukocytes/field; n=6) suggesting that circumferential stretch rather than changes in shear stress was critical to this effect. To investigate the signalling pathways involved in adhesion, we demonstrated that this acute inflammatory response was reduced with the use of inhibitors for membrane integrity (methyl-b-cycloexodrin treatment: control 93±14 treated 47±13; n=4–7; P<0.05). NADPH oxidase (apocynin treatment: 25±3; n=5–7; P<0.05) and mitochondrial permeability (cycloparin A treatment: 50±14; n=4–7; P<0.05) but not altered by inhibitors of xanthine oxidase or cytochrome P450. Increased reactive oxygen species (ROS) production was also seen at 120 mm Hg in HUVECs perfused with the global ROS dyeDCFH (60 nm Hg 3±6 vs 120 mm Hg 149±65 intensity units; n=5–7; P<0.05). We conclude that high intraluminal pressure induces vascular inflammation via circumferential stretch acting on mechno-sensors on the cellular membrane triggering the release of ROS and consequential adhesion molecule expression.

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 acute BP drop ≥20 mm Hg. We used the Finomotor Medi 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 moment 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 mean changes of arm-cuff and Finomotor 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 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 Study (ANBP2 study). ANBP2 was a prospective, open label study with blinded assessment of endpoints designed trial conducted in 6063 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=6064, 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 the 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 (1.19–2.41), 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 invited 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 at 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 (0.4 kg:0.3) and highest (0.2 kg:0.3) tertile. Change in weight predicted change in SBP (P=0.045). Change in SBP differed significantly between the highest and lowest exercise tertile (P=0.033), SBP increased (2.7 mm Hg:2.8) in the lowest tertile and decreased in the highest tertile (~1.4 mm Hg:1.2). Similarly change in weight predicted change in DBP (P=0.003) which differed significantly (P=0.034) between the lowest exercise tertile (0.6 mm Hg:1.0) and the highest (~1.7 mm Hg:0.6). 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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Hypothalamic regions have been shown to have greater neuronal activation in the hypertensive BP/2J mice compared with normotensive BP/3J mice. Gene array studies performed in hypothalamic tissue from Schlagert mice sho the hypothecin gene is upregulated in BP/2J compared with BP/3J mice. Hypothecin or oxin it is alternatively known is shown to be involved in cardiovascular control, sympathetic output, stress response, activity and metabolic state and energy balance. There is a noticeable association between the oxin upregulation in BP/2J mice and previously reported phenotype abnormalities including hypertension, sympathetic over-activity, exaggerated stress response and greater locomotor activity levels. As such it is possible that the metabolic profile of BP/2J mice may also be irregular. To determine whether BP/2J mice have an abnormal metabolic profile, 4 BP/3J and 4 BP/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 resting energy level. Body composition was also measured using EchoMRI. Mice were assessed at 10 and 23 weeks of age to assess changes during development. BP/2J mice showed greater VO2 and VCO2 compared with BP/3J mice at both ages (P<0.0001), yet this declined with age in both species (P<0.0001). Heat production was higher in BP/2J mice at 10 (P<0.0001) and 23 (P<0.003) weeks of age. Total activity level was greater in BP/2J compared with BP/3J at both ages (P<0.0001) indicating greater energy demand but this only declined with age in the BP/3J, whilst the BP/2J activity remained higher. There was a markedly greater food consumption in 10 week old BP/2J mice compared to BP/3J (P<0.0001) which disappeared with age (P=0.4). Yet body weight (BW) was consistently lower in BP/2J compared with BP/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 BP/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 telemetered 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 reductions in HRV or BRS compared with their controls. The SHR exhibited increased BRS in anaesthetised conditions, yet this was not observed in FSL, and this was associated with reductions in HRV. (2) No differences were observed in the control strains or disease models; the only notable reduction observed in the control strains was in HRV in the SHR and FSL under anaesthetic conditions, whilst the FSL exhibited reduced total and high frequency HRV. (3) Reduced HRV was consistent in the LPK and FSL, (2) HRV was similar when 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 anaesthesia 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 ABERANT CARDIAC MICRORNA EXPRESSION IN HERITABLE POLYGENIC CARDIAC HYPERTROPHY

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From soon after birth the Hypertrophic Heart Rat (HRH) normotensive model of human polygenic cardiac hypertrophy has a reduced complement of terminally differentiated cardiomcyocytes, leading to hypertrophy, cardiac failure and premature death in adult rats. 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 normal Heart Rat (NHR). Total RNA was purified from the hearts of postnatal day 2 HH (n=4 males, n=4 females) and NR (n=4 males, n=4 females) using MicroRNAeasy 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 Fus-1. These findings suggest that dysregulation of various microRNAs during the neonatal period may be an important regulatory mechanism governing cardiomyocyte 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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1St Vincent’s Institute of Medical Research, Fitzroy, Victoria, Australia; Departments of 2Pharmacology and 3Medicine, The University of Melbourne, Parkville, Victoria, Australia. 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 the ischaemia-reperfusion. Myocardial infarct size, expressed as the percentage of area at risk, was 40.3% (mean±SEM, n=7) in vehicle-treated rats. Myocardial infarct size was reduced similarly by all 3 treatments; aliskiren: 24.5% (n=5, P=0.038, Dunnnett’s t-test); valsartan: 25.6% (n=6, P<0.04), 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 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 the ischaemia-reperfusion. Myocardial infarct size, expressed as the percentage of area at risk, was 40.3% (mean±SEM, n=7) in vehicle-treated rats. Myocardial infarct size was reduced similarly by all 3 treatments; aliskiren: 24.5% (n=5, P=0.038, Dunnnett’s t-test); valsartan: 25.6% (n=6, P<0.04), aliskiren plus valsartan: 22.3% (n=8, P=0.012). We conclude that aliskiren and valsartan produced similar cardioprotection, without further cardioprotection by their combination. Together with our previous studies, these results suggest that aliskiren is cardioprotective independent of change in circulating and cardiac angiotensin levels, and its cardioprotection may be dependent on the associated increase in cardiac bradykinin and tissue kallikrein expression.

Comparision 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 and various cardiovascular diseases. The aim of this study was to compare the direct method of measuring BP, HR and SNA in rabbits using radiotelemetric measurement in conscious freely 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±0.5°C) conditions. All rabbits received a radiotelemetry implant to monitor BP, HR and RSNA 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 2s of RSNA evoked by 50 mc 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 (89.2 ± 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 nA in caged and lab constrained animals, respectively) were not different 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 impact stress to the animals.

Effects on Cardiac Structure in Adult Rats When Intrauterine Growth Restriction Is Coupled with Hyperglycemia

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We hypothesised that intrauterine growth restriction (IUGR) renders the heart vulnerable to diabetic heart disease. The aim of this study was to examine the effect of induction of hyperglycemia on myocardial collagen deposition and cardiac function in IUGR adult rat offspring. At 24 weeks of age, diabetes was induced in male IUGR and non-IUGR offspring by streptozotocin injection; long-acting insulin was injected daily to maintain blood glucose levels at either a mild level (7–10 mmol/L, n= 24 in IUGR and 24 in non-IUGR controls) or a moderate level (15 –18 mmol/L, n= 12 in IUGR and 12 in non-IUGR controls). Induced hyperglycemia in adulthood was not different between IUGR and non-IUGR adult rats however, levels of cardiac fibrosis were greatest when diabetes was combined with IUGR. Tight glycemic control, in general, attenuated the adverse effects of hyperglycemia but did not reverse the increased collagen deposition. In conclusion, exacerbated fibrosis in diabetic IUGR hearts even when glycemia is tightly controlled may lead to long-term cardiac dysfunction.

Differential Changes in Large Artery Haemodynamics Following Sympathectomy in Normo and Hypertensive Rats

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Arterial stiffness is a predictor of cardiovascular disease and all-cause mortality. Arteries stiffen with age and 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 artery 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 = 9) 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.
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 responses. In the present study we examined the role of nNOS sensitive redox disturbances in the brain NA system play a role in the regulating the cardiovascular response to fear-related stimuli in normotensive radiotatometry-instrumented rats. We found that lentivirus-mediated overexpression of superoxide dismutase 1 (SOD1) in the A1/C1 cell group increases blood pressure responses to conditioned aversive stimuli significantly over control animals (26±1.8 vs 21±1.1 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±1.3 and 96±3.2 mm Hg). Preliminary data 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 display 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 hypertension pathophysiology 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 these differences remain unknown. This study aimed to assess contractile and Ca2+ handling responses in male/female hypertrophic cardiomyocytes subjected 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 hypertrophic cardiopathologies in a hypertension-independent setting. Cardiomyocytes were isolated (enzymatic digestion) from male/female HHR and control Normal Heart Rat (NHR) hearts (12–16 weeks). Myocyte contraction (edge-detection microscopy) and intracellular Ca2+ levels (microfluorimetry, indo1/400x) were tracked in Fura2-AM loaded cells. After 5 mins stabilization (4 Hz, 2.0 mM Ca2+), 37 °C), myocytes were subjected to 20 mins simulated reperfusion (n=6). Ca2+ transient (male 0.67±0.14 vs 0.42±0.05; female 0.54±0.13 vs 0.32±0.05; P=0.048) amplitude were larger in male and female HHR myocytes compared with NHR controls. After 30 mins reperfusion, contraction and Ca2+ loading was equivalent in male NHR/HHR myocytes. In contrast, female HHR myocytes exhibited marked rebound elevation of twitch function post-ischemia, while NHR myocyte recovery was relatively suppressed (% shortening: 7.10±0.76 vs 2.36±0.69; P<0.005), despite both strains exhibiting similar Ca transient amplitudes. This indicates male/female myocytes respond differently to ischemic insult at the electromechanical level, and hypertrophically in females confers a distinctive difference in reperfusion recovery. This suggests sex specific interventions targeted to optimize post-ischemic recovery in the hypertrophied myocardium may be of importance.

THE ROLE OF NITRIC OXIDE IN THE REGULATION OF ARTERIAL PRESSURE IN NEPHRON DEFICIENT MICE

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GDNF heterozygous (HET) mice are born with either 2 small kidneys (HET-2K) and a 30% nephron deficit, or a solitary kidney (HET-1K) and a 65% nephron deficit, compared to wild-type (WT) littermates. Despite these marked reductions in nephron number, GDNF-HET-2K and HET-1K mice are normotensive and show no evidence of renal disease when followed up to one year of age. Recent studies in rodent models of surgical reduction in renal mass have demonstrated that nitric oxide (NO) deficiency must be present for hypertension and chronic kidney disease to develop. Thus this study examined the role of NO in the regulation of conscious arterial pressure in 30 week old WT (n=8), HET-2K (n=6) and HET-1K (n=5) mice with the hypothesis that NO synthase inhibition with LNAME would increase arterial pressure to a greater extent in nephron deficient mice. Basal mean arterial pressure (MAP) was recorded via radio-telemetry for 7 days. LNAME was then given in the drinking water (0.5 mg/ml) and MAP was followed for a further 7 days. Body weights were not different between WT, HET-2K and HET-1K mice. Total kidney weight of HET-2K and HET-1K mice was significantly less than WT (P<0.005). MAP of WT mice remained stable over the 7 day 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±1.1 mm Hg), followed by HET-2K (+11.1±1.7 mm Hg) and least in HET-1K mice (+7.6±2.9 mm 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 nephron 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 AUTOSOMAL 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 function and sympathetic overactivity, but intact baroreflex control of the splanchnic sympathetic nerve. The present study aimed to further investigate the baroreflex pathway by examining both the sensory afferent component of the baroreflex arc and baroreflex control of a different sympathetic nerve bed, the renal nerve. In urethane anaesthetised Lewis polycystic kidney (LPK) and Lewis control rats (total n=23), changes in aortic depressor nerve activity (ADNA) and renal sympathetic nerve activity (RSNA) were recorded with sodium nitroprusside (50–500 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, LPK rats had significantly higher resting heart rate (HR, 351±7 vs 414±10 beats per minute, P<0.0001), systolic blood pressure (SBP, 113±4 vs 174±8 mm Hg, P<0.0001) and RSNA (3.5±0.5 vs 6.6±0.8 μV, P<0.001) but comparable ADNA (0.9±0.2 vs 1.0±0.3 μV). The ADNA barocurve was comparable in LPK 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 splanchnic nerve, baroreflex control of RSNA is impaired in the LPK model, indicative of different perturbations in the sensory afferent and/or central baroreflex mechanisms. Together, these results suggest that impaired sympathetic baroreflexes are due to altered central and/or efferent mechanisms rather than a defect in the sensory afferent pathway of the reflex.

THE PLEITOEIC 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 LEPR 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. 16.8 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 other 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.
NEUROMEDIN U CAUSES BIPHASIC EFFECTS ON SYMPATHETIC VASOMOTOR TONE AND INCREASES RESPIRATORY DRIVE IN RAT ROstral VENTROLUMERAL MEDULLA
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The rostral ventrolateral medulla (RVLM) is the key nucleus for controlling sympathetic vasomotor outflow and also essential for the integration of sympathetic reflexes. Neurormedin U (NMU), a hypothalamic neuropeptide, increases blood pressure and sympathetic nerve activity when injected intracerebroventricularly. However, the central areas that mediate these cardiovascular effects are unknown. The present study was conducted in urethane-anaesthetized, vagotomised and artificially ventilated male Sprague-Dawley rats (n = 16) to investigate the effects of bilateral microinjection of NMU into RVLM on cardiorespiratory variables. Bilateral injection of 25 and 50 pmol of NMU elicited a pressor response, tachycardia, and an increase in splanchic SNA (SSNA) and lumbar SNA (LSNA) at lower doses (25 and 50 pmol). At a higher dose (100 pmol), NMU caused a biphasic response, a brief hypertension and sympathoexcitotoxicity following by a prolonged hypotension and sympathoinhibition. The peak excitatory and inhibitory response was found at 100 pmol NMU with an increase in MAP, HR, SSNA and LSNA of 33 mm Hg, 25 bpm, 42% and 52%, respectively, from baseline. NMU in the RVLM, also increased phrenic nerve amplitude, expiratory period, and reduced inspiratory period. The AUC of inspiratory I peak of both SSNA and LSNA are potentiated at the excitatory phase of NMU response in the RVLM, but at inhibitory phase the I peak of both SNA are reduced. On the other hand, the AUC of post-inspiratory peak of both SSNA and LSNA is reduced at excitation as well as inhibitory phase of NMU effect. The present study provides functional evidence for a complex differential modulatory activity of NMU on the cardiovascular and respiratory responses that are integrated in the RVLM.

THE EFFECT OF PERINDOPRIL ON THE INTRA-RENAL RENIN ANGIOTENSIN SYSTEM AND CIRCULATING CATECHOLAMINES IN POLYCYSTIC KIDNEY DISEASE
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Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia. Hypertension is a common presenting factor in Poly cystic Kidney Disease (PKD) patients prior to the onset of renal failure. The renin-angiotensin system (RAS) is a key regulator of blood pressure, through both its direct actions and its influence on the sympathetic nervous system (SNS), both of which may contribute to hypertension in these patients. Circulating angiotensin II (Ang II) levels in hypertensive PKD patients are often normal or reduced, however angiotensin converting enzyme (ACE) blockade is an effective anti-hypertensive therapy. Up-regulation of intra-renal RAS has therefore been postulated as a mechanism driving hypertension in PKD. This study examined if chronic ACE inhibition has influences on intra-renal RAS gene expression and plasma catecholamine levels as an indicator or baseline SNS activity in a rat model of PKD, the Lewis Poly cystic Kidney (LPK) rat. LPK rats were treated with perindopril (3mg/kg/day/po) from 4 – 12 wks of age. Systolic blood pressure was measured weekly via tail cuff plethysmography. Animals were euthanased at 12 weeks and kidneys collected for quantitative RT-PCR for RAS genes (angiotensinogen, renin, ACE I & II) and the ATRIA Blood was collected for assessment of renal function (serum urea and creatinine) and determination of circulating catecholamine levels by HPLC. Chronic perindopril treatment resulted in a significant reduction in blood pressure in treated LPK (164 ± 13 mm Hg) vs. untreated LPK (226 ± 10 mm Hg) P < 0.0001. Intra-renal levels of RAS genes were 2 and 2.6 fold greater in LPK treated animals for renin (P = 0.009) and ATRIA (P = 0.02) respectively, but unchanged for the other RAS genes studied. Plasma levels of noradrenaline (Na) and adrenaline (A) were significantly less in the LPK treated (Na = 183 ± 66 pg/ml, A = 220 ± 105 pg/ml) vs. untreated LPK (Na = 1257 ± 549 pg/ml, A = 1608 ± 272 pg/ml) (P < 0.0001 and P < 0.0032) respectively. Serum markers of kidney dysfunction were unchanged between groups. The increase in intra-renal mRNA for renin and renin-like mRNA in LPK rats correlated well with hypertension and enhanced SNS sensitivity responses. The reduction in blood pressure and reduced levels of circulating catecholamines after treatment with perindopril suggest a relationship between Ang II and SNS activity.

INHIBITION OF CHOLESTEROL ESTER TRANSFER PROTEIN IN HEALTHY HUMANS INCREASES CIRCULATING INSULIN, INSULIN SECRETION AND CHOLESTEROL EFFLUX FROM PANCREATIC ß-CELLS
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High-density lipoprotein (HDL)-raising agents currently in development for coronary disease may also have efficacy for the management of type 2 diabetes mellitus. We have previously shown that HDL and apoA can promote insulin secretion from pancreatic ß-cells and actively increase cholesterol effluxing from ß-cells. The current study assessed the effects of elevating HDL and its major associated protein, apolipoprotein A1 (apoA1) via a cholesteryl ester transfer protein inhibitor (CETPi) on postprandial insulin, insulin secretion and cholest erol efflux from MIN6N8 ß-cells. Healthy participants received a second generation CETPi (RG7232–F. Hoffman Laroche) n = 10 or matching placebo (n = 15) for 14 days in a randomized, double-blind study. CETPi inhibition resulted in 80% inhibition of CETP activity, with increased circulating HDL (0.55 ± 0.06 mmol/L) and associated apoA1 (26.7 ± 4.7 mg/dl) (all P<0.001 vs placebo). There was also a greater increase (P<0.05) in postprandial plasma insulin after CETPi treatment (14±1.55 μU/ml) than placebo (22±3.58 μU/ml) over the 14 days. MIN6N8 ß-cells pre-treated with oxidized low-density lipoprotein (oxLDL) and plasma from CETPi-treated individuals increased both acute (CETPi: 122±106 μU/ml vs placebo: 228±7.8 ng/ml/hr) and chronic CETPi: 37±20 vs placebo: 61±18 ng/ml/hr) in vitro glucose-stimulated insulin secretion (GSIS) over the treatment period (P<0.05), with no effect on basal insulin secretion. Increased insulin secretion over the 14 days was associated with a 10 fold increase in the capacity of CETPi-plasma compared to placebo-plasma to support cholesterol efflux from MIN6N8 cells (P<0.05) over the same period. Collectively these data suggest that CETP inhibition increases postprandial insulin responses in humans through HDL-apoA1-mediated stimulation of acute and chronic GSIS. These effects of CETPi may be mechanistically linked to enhanced cholesterol efflux from ß-cells. Whether CETPi inhibition mitigates against harmful post-prandial glucose elevations after chronic therapy warrants further investigation.
ASSOCIATION OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH TYPE II DIABETES AFTER ACUTE CORONARY SYNDROME

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Patients with type 2 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. While 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 Hospital Heart 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 and LDL cholesterol levels were well within the normal range, in the patient cohort, systolic blood pressure was dependent responses to acetylcholine (0.92, 18.5, and 37 ± µg/ml/min), measured using venous occlusion strain gauge plethysmography were significantly decreased in T2D + ACS patients compared to controls (n = 13–14; P = 0.046) while endothelium-independent responses to sodium nitroprusside were not (n = 14–15; P = 0.142). Similarly, the reactive hyperemic index (RHI), a measure of endothelial dysfunction using reactive hyperemia peripheral arterial tonometry was decreased in the T2D + ACS study group when compared to controls (1.75 ± 0.17 vs 2.43 ± 0.33 respectively; n = 15–16; P = 0.04). Endothelial microparticle (EMP) numbers have also been shown to increase and correlate 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.

ANALYSIS OF IMPACTS OF ENDOTHELIAL FUNCTION ON BLOOD PRESSURE REGULATION AND STROKE RISK IN HIGH FAT FED RABBITS

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Aldosterone is thought to be an important contributor to hypertension. Moreover, there is an association between high aldosterone levels and the risk of stroke and transient ischemic attack that is independent of blood pressure and other risk factors. It is also known that patients with congestive heart failure and diabetes have more strokes than those with heart failure alone, 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 (ACl; endothelium-dependent agonist) compared to vehicle, suggesting that aldosterone causes endothelial dysfunction. Aldosterone had no effect on ACh responses in Nox2−/−mice. Subcutaneously administered cerebral oxidative stress was assessed, and baseline and Nox2-stimulated superoxide levels were higher in cerebral arteries of aldosterone vehicle-treated and 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.

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

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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, hypertension 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) intracerebroventricularly 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, rabbits on HFD and normal fed rabbits were similar in both BP and RSNA, with no significant changes in heart rate or blood pressure. These data suggest that the rapid changes in BP, HR and RSNA were brought about by peripheral mechanisms. These changes appeared to be associated with pathways mediating the sympathetic responses to acute emotional stress that are independent of baroreflex or chemoreflex pathways.

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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 types. Up-regulation of Nox4 is known to induce angiogenesis in endothelial cells, whereas in fibroblasts it increases collagen production and promotes fibrosis. Prostacyclin (PGI2) is long known to be cytoprotective, but this signaling interacts 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 proliferation, migration, inflammation, and extracellular matrix (ECM) production. Stimulation of Nox4 induces cell proliferation and collagen production. These effects in both cell types were blocked by either adenovirus-mediated Nox4 RNAi (Ad-Nox4) or dominant- negative Nox4 (Ad-DNNOx4). TGF-β induced in polyvinyl alcohol sponges implanted subcutaneously significantly (n = 6; P < 0.01) increases angiogenesis, indicated by percentage vascular volume (CD31+ vessels) and collagen content (fibrosis) in C57BL/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 endothelial cells that the Nox4 antagonist acetoxycoumarin inhibited 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; 2BakerID Heart and Diabetes Institute, Melbourne, Victoria, Australia; 3EM, Université Paris VII, Paris, France; 4Réanimation pédiatrique, Hôpital Necker-Enfants Malades, Université Paris V, France. Endothelial inflammation and leukocyte/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) was measured by dichlorofluorosceindiacetate (H2DCFH-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 294002. The levels of ceramide, and expression of ICAM-1, were measured in HUVECs. We studied the effect of IL-10 on leukocyte/endothelium interaction induced by TNF-α using an ex vivo perfused vessel model. IL-10 significantly reduced H2DCFH-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 + LY: 12.5% / + 2.3; TNF-α / IL-10 vs TNF-α / IL-10 + Wort: 86.7% / + 16.3; TNF-α / IL-10 + Wort vs TNF-α / IL-10: 4.44 / + 1.1 to 50.21 / + 12.41 at 60 min). Pre-treatment with IL-10 significantly decreased the levels of ceramides in HUVECs (TNF-α / vs TNF-α / IL-10: 6278 / + 1013 vs 1440 / + 128.1 pmoles/mg prot. IL-10 significantly decreased ICAM-1 expression and leukocyte adhesion (TNF-α / vs TNF-α / IL-10: 26.80 / + 2.66 vs 6.75 / + 0.38 adherent leukocytes/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 (Rainie) 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 < 0.005 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 the slope of this 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 rising is 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 phenylephrine 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 nitropussite. Aortic PW and thoracic MAP were recorded over the entire pressure range. A maximal directional dependence was observed at MAPs of 70 mm Hg and 120 mm 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 level 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 Waves 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 resistive properties. 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 (MCAF) with transcranial Doppler under control conditions and physiological challenge (Valsalva). Aortic pressure (PA) 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 (PxA), and in the frequency domain as vascular impedance. Mean MCAF was relatively high (6osis < 14 cm/s) and AP 86 ± 15 mm Hg, but within normal limits. PxA was 84 %, and PxA 11 %, also within normal limits for age, but with PxA lower and FAx higher than in older subjects with suspected and confirmed cerebrovascular disease. Impedance modulus was 1.8x10^3 dynes/cm 2 at zero Hz and fell to an average of 0.3x10^3 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 regardless in the region downstream the cerebral vessels which is consistent with an overall low 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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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 Australian 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 practitioners, conducted between October 2009 and November 2010 of 293 persons with cardiovascular disease (CVD) or at risk heart (3 or more CVD risk factors) of said. We identified symptomatic individuals by use of the Edinburgh Claudication Questionnaire. Multivariable
HOW RELIABLE IS VISIT-TO-VISIT CLINIC 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 patients 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±17 SD years). Mean BP (±SD) did not significantly vary between the 2 clinic visits (144±20/80±11 vs 145±19/80±10 mm Hg) and was similar to 24 hour awake BP (135±19/78±9 mm Hg). Pearson’s correlation of mean clinic SBP with mean 24 hour awake SBP confirmed this agreement (Fig. 1, r=0.73, P<0.05). It is therefore important that BP variability for the individual patient is identified to tailor treatment.

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 0.97–2.97, 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=0.03; 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 hypertension.

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Figure 1. Pooled HR of CV events and mortality per 10 mm Hg increase in exercise systolic BP adjusted for resting BP, age and CV risk factors.
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 randomly assigned to either active treatment (perindopril) or matching placebo(s). The outcomes were estimated using linear or logistic regression analysis. Median time from onset (1-2 min) to IPC generation was 5 min. IPC was induced by 3 cycles of 5 min femoral artery occlusion interspersed with 5 min reperfusion. Rats subjected to IPC generated bigger tissue constructs at 7 and 28 days (34% and 75% increased, respectively). The analyses of achieved follow-up BP levels showed that the lowest risk of intracranial bleeding was observed among participants who achieved the lowest follow-up systolic BP levels (median 113 mmHg). 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 trolox-A (TSA) (1 ng/mL) during embryoid body formation yielded beating CMs that expressed several key cardiac markers, cyclin cycled and were responsive to chronotropic agents (isoproterenol 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 selected to minimize the risk of teratoma formation. In a proof-of-concept study, we used a fluorescent mitochondrial dye, tetramethylrhodamine methyl ester perchorlate (TMRM), to sort cells by FACs, resulting in two populations of neonatal rat CMs. The TMRM-high cells consisted of trolox-positive CMs that maintained spontaneous contractions for 6 months while TMRM-low cells were non-contractile and trolox-negative. Therefore, TMRM staining and sorting suggests a viable method to enrich hPSC-derived CMs. In conclusion, we have presented multiple strategies (cryopreservation with IPC, cardiac differentiation with small molecule TSA, and CM enrichment with TMRM) 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 precede eclampsia and is defined as hypertension in pregnancy accompanied by proteinuria. This condition is associated with significant maternal and fetal morbidity and mortality. PE has been linked to multiple biomarkers of inflammation, notably pro-inflammatory chemokines, cytokines and adhesion molecules, but the underlying pathogenesis of PE is poorly understood. PE is strongly associated with other vascular diseases.

Pregnancies affected by PE and IUGR are characterised by poor placental development and a failure of normal placentation or causing vascular dysfunction. Immunostaining of placental biopsies indicates that the human-specific TP isoform is more abundant in PE placentas than in normal placentas. Although the human-specific TP isoform has a similar approach of vascular dysfunction, the contribution of the human-specific TP isoform to PE remains unclear. Here we present multiple strategies (cytoprotection with IPC, cardiac differentiation with small molecule TSA, and CM enrichment with TMRM) that advance us towards generating transplantable human cardiac constructs.

human amnion stem cells improve post-stroke outcome in mice

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1Department of Pharmacology, Monash University, Clayton, Victoria, Australia; 2The Ritchie Centre, Monash Institute of Medical Research, Clayton, Victoria, Australia. Stem cells derived from human tissue, including embryonic, induced pluripotent, neural, and mesenchymal cells, have already been reported to rescue injured brain tissue and improve functional recovery in experimental models of stroke. However, there are potentially major limitations to each of these sources of stem cells that may ultimately prevent their use in a viable mainstream treatment option for stroke patients. Conversely, stem cells derived from the placenta, called human amnion epithelial cells (hAECs), appear to offer several important advantages over other stem cell lineages, but so far they have received almost no attention as a stem cell source. Thus, we sought to determine if hAECs may be an effective form of cell-based therapy for stroke. To test this hypothesis, male C57Bl/6 mice were subjected to 0.5 h middle cerebral artery occlusion-reperfusion. Mice were then injected with 1x10⁵ fluorescently-labelled hAECs or saline (vehicle) i.v. at either 1 h (acute treatment) or at 72 h (delayed treatment) post-stroke and brains were removed after 3 or 14 days, respectively. Neuronal and motor assessment tests were performed every 3–4 days. hAECs injected acutely after stroke were evident at 72 h in the ischaemic, but not the contralateral cerebral hemisphere. Importantly, neurological and motor function as well as infant death was significantly improved in mice treated with hAECs vs. vehicle 1 h post-stroke (n=7–12; P<0.05). Furthermore, immunofluorescence of the key marker of apoptosis, cleaved caspase-3, revealed that less apoptosis occurred in the infarct core of mice treated with hAECs (P<0.05). Moreover, mice that received delayed post-stroke hAEC treatment had improved survival to 14 days compared with vehicle-treated mice, although functional outcomes were not different between surviving animals from each treatment group (n=10–group). Thus, early-post-stroke i.v. delivery of hAECs appears to reduce brain injury and functional delay, and delayed post-stroke hAEC treatment improves survival to 14 days. These findings suggest that hAECs could be a viable and effective clinical therapy for promoting recovery following ischaemic stroke.

NITROXYL SUPPRESSES CEREBROVASCULAR REACTIVE OXYGEN SPECIES GENERATION AND CONSTRICCTOR RESPONSES TO ANGIOTENSIN II VIA INHIBITION OF NO2-NADPH OXIDASE

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Elevated production of reactive oxygen species (ROS) by NO2-NADPH-oxidase is an important underlying cause of cerebrovascular dysfunction in several vascular diseases. Here we tested the hypothesis that nitroxyl (HNO), a novel redox form of nitric oxide (NO) with therapeutic advantages over NO, limits ROS levels and constricctor responses to angiotensin II (Ang II) in the cerebral circulation via inhibition of Nox2-NADPH oxidase. The effect of the HNO donor, isopropylamine NONOate (IPA/NO), was studied on Ang II (0.1 μM)-stimulated superoxide (O2·-) generation (lucigenin 5 μM-enhanced chemiluminescence), Ang II- and PDB (1 μM)-stimulated hydrogen peroxide (H2O2) generation (Amplex Red fluorescence) and Ang II (1nm-1μM)-mediated constriction (perfusion myography) was examined in cerebral arteries (pooled basilar and middle cerebral) from male C57BL/6 wild-type (WT) mice. Ang II-stimulated O2·- generation by cerebral arteries from WT mice (150±25–10⁴ counts/s/mg; n=11) was suppressed in a concentration-dependent manner by IPA/NO such that 1μM decreased levels by 52±3% (n=13; P<0.05). IPA/NO (1 μM) also suppressed Ang II- and PDB-stimulated H2O2 levels in cerebral arteries by ~40% (n=11–13; P<0.05). The ability of IPA/NO (1 μM) to decrease O2·- levels was reversed by the HNO scavenger L-cysteine (3mM; vehicle 1 L-cysteine, 98±21; IPA/NO 1 L-cysteine, 100±8; 10² counts/s/mg; n=8; P<0.05), yet sustained in the presence of the NO scavenger hydroxocobalamin (100 μM; n=7) and stiG inhibit ODQ (10 μM, n=6). IPA/NO did not inhibit Ang II-stimulated O2·- production by cerebral arteries from Nox2-deficient mice (vehicle 103±10 vs IPA/NO: 102±9×10⁴ counts/s/mg; n=5). Moreover, IPA/NO (1μM) abolished Ang II-induced contractions of middle cerebral arteries from WT mice (Δ diameter for 1 μM Ang II: Control – 13.3%; IPA/NO 1:0.6%; n=5; P<0.05) without affecting constricctor responses to high K+ (124 mM; n=5) or the thromboxane A2 mimetic U46619 (n=5). In summary, HNO rapidly suppresses Ang II-stimulated ROS generation in mouse cerebral arteries via cGMP-independent inhibition of Nox2-NADPH-oxidase. Such an action leads to attenuation of AngII-induced contractions of the middle cerebral artery in vitro. An ability of HNO to suppress ROS generation by Nox2-NADPH oxidase may facilitate the use of HNO in the treatment of cerebrovascular dysfunction associated with hypertension and other vascular diseases.

angiotensin AT1a receptor knockdown attenuates thepressor response to PAH/endothelin but not ANG II in the mouse

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Brain angiotensin AT1 receptors have been implicated in modulating cardiovascular reactivity to averse stimuli associated with imminent danger, such as restraint or footshock. However, the involvement of AT1 receptors on cardiovascular reactivity to angiotensin (distant potential stimulus) remains unknown. In the current study, we examined the effects of knockdown of angiotensin related to go to a new page.

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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 TA/TA mice. Conversely, in response to a panicogenic stimulus, footshock, AT(1A) receptor knockout mice showed decreased flight (24%, P < 0.01) and pressor reactions (32%, P < 0.002). Likewise, AT(1A) receptor mice displayed reduced flight behaviour (escape from the open arm) in the elevated T-maze test (24%, P < 0.02). During re-exposure to the context (footshock chamber), AT(1A) receptor knockout mice also showed decreased freezing and pressor responses. In AT(1A) receptor mice, the re-exposure-induced c-fos expression was markedly (10-fold) attenuated in the periaqueductal grey, a midbrain area that is critical for mediating emotional responses, whereas c-fos expression in the hypothalamic nucleus 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(1A) receptors may thus be a potential target to selectively control pre- and behavioral components of panic-like reactions, without compromising general cardiovascular reactivity and risk assessment behaviour.

61 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 in clinical trials 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 ET-1 effects in pulmonary vessels. The aim of this study was to investigate the efficacy of bosentan, an ET(A)/ET(B) receptor antagonist, on endothelin-1 induced contractions in rat pulmonary arteries. These results suggest that ET(A) receptor antagonists may be effective in the treatment of pulmonary hypertension.

62 THE EFFECT OF CHRONIC TREATMENT WITH DIAZEPAM ON STRESS AND HYPERTENSION IN SCHLAGER MICE

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Bosentan (1–100 μM) is 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 investigated in rat pulmonary arteries. In absence of CGRP bosentan (1–100 μM) induced contractions in isolated human radial and pulmonary arteries collected from coronary bypass and lung resection surgery. ET-1 (0.1–300 nM) caused concentration-dependent right-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. In human radial and pulmonary arteries, the ET-1 concentration-response curve was significantly right-shifted by bosentan (1 μM, P < 0.05 vs. vehicle control). In ET-1 pre-contracted rat mesenteric and human radial arteries CGRP (0.1–300 nM) caused concentration-dependent relaxation (68 ± 4%, but was unable to relax either rat or human pulmonary arteries. These results 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.

63 IMPORTANCE OF THE MEDIA-MYOGYRIA TO THE SYMPATHETICALLY MEDIATED HYPERTENSION IN SCHLAGER MICE

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Schlagter BPH/2J mice are hyporesponsive due to an overactive sympathetic nervous system (SNS) and are known to have an exaggerated blood pressure (BP) reactivity to stress. The medial amygdala (MeAm), a major forebrain region integrating the autonomic response to stress, was found to have the highest correlation with both active (night) and inactive (day) blood pressure levels in Schlagter mice. Additionally, the MeAm was the only region to show greater neuronal activation in BPH/2J mice in the daytime inactive period compared with normotensive (BPH/3J) mice. To establish whether the MeAm could be contributing to the hypertension, we assessed the effect of AT(1A) receptor ablation and BPH/3J mice were implanted in 10 normotensive (BPH/3J) and 8 hypertensive (BPH/2J) mice. Two weeks later, ischemic (10mg/ml) was injected bilaterally into the MeAm to lesion this region. In the week before and 3 weeks after lesion surgery, cardiovascular parameters were measured for 48 hours and a series of stress tests were performed. During control measurements, BP was 121 ± 44 mm Hg in BPH/2J compared with 101 ± 22 mm Hg in BPH/3J mice (P < 0.0001). Discrete lesions that reduced MeAm neuron number by 77% (n = 7) caused a lower BP in BPH/2J compared with BPH/3J mice, in the inactive (P < 0.05) and active periods (P < 0.004), suggesting that the lesion reduced SNS activity. Despite the reduction in BP in BPH/2J mice, the rescuer response to stress was maintained in BPH/2J lesion mice. Furthermore, the pressor response to cage change was greater than prior to lesions in BPH/2J mice (P < 0.02). Thus the MeAm seems to provide a tonic contribution to BP, which is independent of its role in stress reactivity or circadian BPH influences. These results clearly show that the MeAm is important to the hypertension in Schlagter BPH/2J mice, most likely via activation of the SNS.

64 REGIONAL BIOMECHANICAL PROPERTIES OF THE AORTA IN POLYCYSTIC KIDNEY DISEASE RATS

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Aortic stiffness and composition are important indicators of cardiovascular risk as they may predict the risk of cardiovascular disease, further compromising blood pressure regulation and increasing 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 descending thoracic (TA) and abdominal aortas (AA) were isolated from 12-week-old male rats. Aortic rings were assessed in vitro using a uniaxial tensile test in which the circumferential 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 (44 ± 2 vs 55 ± 4%, 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 only (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 ± 0.01 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 mean values between the groups. The results suggest 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.

65 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 31P para-aminomphorizide clearance techniques. Body weight at 32 weeks was significantly attenuated whereas the females were still exposed to maternal protein restriction. After adjusting for 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 hypertertension in the growth-restricted offspring with GFR/kg weight and filtration fraction all significantly 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 vascular pathology and the incidence of coronary heart disease. Both conditions show 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 (20%), suggesting an interaction between Nox2 and VEGFR was involved in retinal vessel growth in OIR. To confirm our findings, immunohistochemical localisation of Nox2 and VEGF proteins, ROS and endothelial cells in OIR were investigated. In conclusion, Nox2 is an important signalling mediator involved in retinal vascularisation, 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 AVERSIVE 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 showed higher levels of risk assessment behaviour (stretched-attend postures) in food presentation tests. These data suggest that systemic GPX1 inhibition in mice increases the behavioural response, but not cardiovascular reactivity, to aversive 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.

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) varied. ICP was higher than arterial pressure to establish 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 mm Hg, mean ICP 0.5 ± 3.7 mm Hg, pulse AP 60 ± 13 mm Hg, pulse ICP 6.2 ± 2 mm Hg. 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 waveform frequencies in both time and frequency domains indicates ICP pulsations are due to pulsations of pressure in cranial arteries without any appreciable effect of venous pressure. Since aortic pressure pulsations are markedly affected 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 frequently 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-anaesthetised, vagotomised and mechanically ventilated Sprague-Dawley rats, we investigated the effect of ten episodes of 90% O2-10% N2 on sympathetic nerve activity (sNA, and the hypoxic sympathetic chemoreceptor and baroreceptor reflex 60 mins 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 hypoxic chemoreceptor reflex was enhanced (peak change from 92 ± 22 mm Hg; 186 ± 38%; baseline vs. after AIH, P < 0.05), and the sympathetic baroreceptor reflex sensitivity was increased (Gain tmax from 1.79 ± 0.18 to 2.60 ± 0.28 mmHg Hz-1, n = 8, P < 0.05). Pre-treatment with systemic methysergide (40mg/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 hypoxic sympathetic chemoreceptor reflex 60 mins after AIH was unaffected by methysergide pre-treatment (peak change from 150 ± 41 to 231 ± 57%; baseline vs. after AIH, P < 0.05), while the increase in sympathetic baroreceptor reflex sensitivity was abolished (Gain tmax, from 3.16 ± 0.71 to 2.32 ± 0.50 mmHg Hz-1, n = 6, P < 0.05). Our findings indicate that the AIH-induced sympathetic LTF requires 5-HT receptors, however, the enhancement of the hypoxic sympathetic chemoreflex is likely mediated by -nonserotinergic mechanisms.

RENA L SYMPAT HETIC 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 <60 ml/min/1.73m2, 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 hypoxic sympathetic chemoreceptor reflex 60 mins after AIH was unaffected by methysergide pre-treatment (peak change from 150 ± 41 to 231 ± 57%; baseline vs. after AIH, P < 0.05), while the increase in sympathetic baroreceptor reflex sensitivity was abolished (Gain tmax, from 3.16 ± 0.71 to 2.32 ± 0.50 mmHg Hz-1, n = 6, P < 0.05). Our findings indicate that the AIH-induced sympathetic LTF requires 5-HT receptors, however, the enhancement of the hypoxic sympathetic chemoreflex is likely mediated by -nonserotinergic mechanisms.

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