Abstracts From the 31st Annual Scientific Meeting of the High Blood Pressure Research Council of Australia

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001 PHOTOSTIMULATION OF CHANNELRHODOPSIN2-TRANSFECTED C1 NEURONS ACTIVATES PERIPHERAL SYMPATHETIC VASOMOTOR AFFERENTS AND NEURONS IN THE LOCUS COERULEUS AND A5 REGION IN SPRAUGE DAWLEY RATS

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The C1 neurons of the rostral ventrolateral medulla (RVLM) innervate sympathetic preganglionic neurons in the spinal cord that convey sympathetic vasomotor tone, and the catecholaminergic neurons of the locus coeruleus (LC) and A5 region. In this study we investigated the effects of C1 neuron stimulation on sympathetic vasomotor discharge and the activity of LC and A5 neurons. We used a lentivirus that expresses a fusion protein between the light-sensitive Channelrhodopsin 2 (ChR2) and the fluorescent reporter mCherry under the control of the PRSx8 artificial promoter (a Phox2-responsive element) to drive ChR2-mCherry expression selectively into Phox2b-positive neurons (89 ± 1% N = 12). Virus microinjections into the C1 region produce ChR2-mCherry expression in C1 neurons and non-C1 Phox2b-positive neurons in the retrotrapezoid nucleus (1:1 ratio of C1: non-C1 neurons, 70% of all C1 neurons). Pulsed photostimulation of the RVLM with 473 nm laser light (LL) in vivo (10 ms pulses, 20 Hz, 30 s) increased arterial pressure (AP) by 13.8 ± 2.2 mmHg and splanchic sympathetic nerve discharge (s SND) by 60.3% (N = 9). Following chemical blockade of the sympathetic baroreflex, photostimulation produced a significantly greater increase in arterial pressure (P < 0.01; N = 6) and SND (P < 0.05; N = 6). Single LL pulses produced a massive evoked burst of SND (peak; 1443 ± 223% relative to baseline, onset latency: 28 ± 1 ms; N = 9) followed by a long-lasting reduction of SND (18.9 ± 5.5% relative to baseline). Twin-pulse stimulation revealed a significant reduction in the amplitude of the second pulse if delayed less than 2 s after the initial pulse. Low frequency photostimulation of the C1 region produced a temporally precise activation of both LC (16/22; N = 4) and A5 (3/8; N = 2) neurons. Intradecerebroventricular administration of kynurenic acid blocked C1 evoked excitation of LC neurons (12/12). These results confirm that the C1 neurons are sympathetic-corticatory neurons and control AP, but are strongly gated by the sympathetic baroreflex and the properties of sympathetic preganglionic neurons. Furthermore, preliminary data suggests that C1 neurons excite neurons in the A5 and LC, probably through the release of excitatory neurotransmitters.

002 ACUTE ADMINISTRATION OF CHLORogenic ACID REDUCES BLOOD PRESSURE IN THE RAT

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Hypertension is a major risk for cerebro- and cardio-vascular diseases. Although elevated blood pressure (BP) is usually controllable by therapeutic means, diet based strategies to lower BP are becoming increasingly popular, as they may act as a safer and more cost-effective alternative when compared to conventional drug therapy. Recent efforts have identified potential therapeutic roles in human health for specific dietary components (bioactives). For example, several bioactive peptides which inhibit ACE and/or antagonise the AT1 receptor have been isolated, and products consequently formulated. In addition, certain polyphenolics and flavonoids have been shown to reduce BP via mechanisms involving ACE and/or the vasculature. We assessed the effects of chlorogenic acid (CGA), a polyphenol found in coffee, on BP in the adult SHR over a 48 h period, following administration of a single oral dose. Compared to saline control, CGA caused both dose- and time-dependent reductions in BP over the 48 h monitoring period. CGA at 75 mg/kg lowered BP (P < 0.05 or better) at 3, 6, 9 and 24 h post administration. At 250 mg/kg, lower BP was still evident at the 48 h time point (237 ± 1.4 vs 223 ± 2.0 mmHg, P < 0.01). The greatest reduction in BP was observed at the 3 h - (18–20%; 75 mg/kg and 125 mg/kgk) and 6hr (21%; 250 mg/kg) time points. In comparison, Enalapril (1mg/kg) reduced BP in the 3–9 h time window (8–12% vs control), with BP values returning to baseline by the 24 h point. In isolated aortic rings pre-contracted with phenylephrine (PE, EC 50), CGA caused dose-dependent relaxation, however in the PE pre-contracted perfused-mesenteric vascular bed, CGA exerted no relaxation but instead resulted in a paradoxical increase in contraction. In summary, the acute BP model is an appropriate tool to evaluate the potential antihypertensive effects of bioactives. Results also suggest that BP lowering actions of CGA are likely to be mediated via mechanisms other than the lowering of peripheral vascular resistance.

003 AMPLIFICATION OF THE PRESSURE WAVE IN HUMAN UPPER LIMB

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In volunteers for the Asklepios study, Segers et al [Hypertension 2009;54: 414] described brachial-radial amplification as responsible for most of the amplification of the arterial pressure wave in the upper limb. However, the high amplification in the forearm was different to that recorded from invasive studies in the upper limb by Earl Wood in the 1950s and many investigators since. Since findings from Asklepios study has not been seen in other studies, we repeated the study by calibrating the central aortic pressure (generated from the radial waveform), from both the radial artery waveform and from the brachial waveform, recorded by applanation tonometry. We set systolic peak and diastolic nadir of each to cuff brachial systolic (SP) and diastolic (DP) pressure. We then measured the carotid waveform by applanation tonometry, and calibrated this by assuming that mean (MP) and DP were the same as in the arm.

Studies were undertaken in 70 (27 women) volunteers and patients (mean age 70 years SD 14.8) at a clinic site.

Results were broadly in line with the Asklepios study, with amplification of the pulse greatest in the forearm (8.8 (SD 7.5) mmHg, and negligible in the carotid-brachial segment (0.3 mmHg SD 6.4). MP calculated from arithmetic integration of the pressure wave over 8 to 10 cycles enabled determination of Form Factor (FF) – the proportion of pulse pressure needed to be added to diastolic pressure for generation of mean pressure. FF (%) was virtually identical in the brachial artery (39.3 SD 5.1) and carotid artery (39.7 SD 3.9), and quite different to that for the radial artery (34.5 SD 3.9). The brachial waveforms recorded by tonometry were different to the radial, with a blunt peak, and different to waveforms measured invasively in previous studies. Applanation of the brachial artery against bone could not be ensured through the bicipital aponeurosis. High values of FF for the brachial and carotid artery were similar to those of Asklepios group, and correspond to the low amplification of the pulse between carotid and brachial sites.

The current method of calibrating the radial waveform to brachial cuff pressure is more appropriate than using brachial tonometry. Invasive studies of simultaneously recorded brachial and radial waveform will be necessary to confirm or refute this view.

004 ESTIMATION OF AORTIC FLOW FROM DERIVED AORTIC PRESSURE WAVEFORMS: A MODELLING STUDY

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The double TF method (pressure – pressure then pressure – flow), is physiologically realistic, with peak flow corresponding to the first systolic peak pressure, and with flow essentially zero throughout diastole – as in the Doppler waveform. Flow waveforms in the upper limb were realistic, with peak flow corresponding to the first systolic peak pressure, and with flow essentially zero throughout diastole – as in the Doppler waveform. Flow waveforms at different ages were realistic, with peak flow corresponding to the first systolic peak pressure, and with flow essentially zero throughout diastole – as in the Doppler waveform. Flow waveforms from the literature and incorporated into a model which used this as age-dependent TF so as to convert aortic pressure harmonic moduli to corresponding aortic flow moduli; the flow wave in the upper limb. However, the high amplification in the forearm was different to that recorded from invasive studies in the upper limb by Earl Wood in the 1950s and many investigators since. Since findings from Asklepios study has not been seen in other studies, we repeated the study by calibrating the central aortic pressure (generated from the radial waveform), from both the radial artery waveform and from the brachial waveform, recorded by applanation tonometry. We set systolic peak and diastolic nadir of each to cuff brachial systolic (SP) and diastolic (DP) pressure. We then measured the carotid waveform by applanation tonometry, and calibrated this by assuming that mean (MP) and DP were the same as in the arm.

Studies were undertaken in 70 (27 women) volunteers and patients (mean age 70 years SD 14.8) at a clinic site.

Results were broadly in line with the Asklepios study, with amplification of the pulse greatest in the forearm (8.8 (SD 7.5) mmHg, and negligible in the carotid-brachial segment (0.3 mmHg SD 6.4). MP calculated from arithmetic integration of the pressure wave over 8 to 10 cycles enabled determination of Form Factor (FF) – the proportion of pulse pressure needed to be added to diastolic pressure for generation of mean pressure. FF (%) was virtually identical in the brachial artery (39.3 SD 5.1) and carotid artery (39.7 SD 3.9), and quite different to that for the radial artery (34.5 SD 3.9). The brachial waveforms recorded by tonometry were different to the radial, with a blunt peak, and different to waveforms measured invasively in previous studies. Applanation of the brachial artery against bone could not be ensured through the bicipital aponeurosis. High values of FF for the brachial and carotid artery were similar to those of Asklepios group, and correspond to the low amplification of the pulse between carotid and brachial sites.

The current method of calibrating the radial waveform to brachial cuff pressure is more appropriate than using brachial tonometry. Invasive studies of simultaneously recorded brachial and radial waveform will be necessary to confirm or refute this view.
MEASUREMENT OF MEAN ARM PRESSURE FOR CALCULATION OF CENTRAL AORTIC SYSTOLIC AND PULSE PRESSURE

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In 1992, Kelly and Fitchett [JACC 1992;20:952] proposed that the carotid pressure waveform, measured non-invasively by applanation tonometry, could be calibrated from upper arm mean and diastolic pressure, with the assumption that these values were identical in central and peripheral vessels. This has been widely used since but the practice has been questioned when mean pressure (MP) is calculated from diastolic pressure plus a fixed proportion (Factor FF) of systolic pressure (PP) [Hypertension 2009;54:414].

This study was undertaken to establish in a clinical database, the range of FF in tonometric radial artery waveforms, and in aortic waveforms calculated from a generalised aortic-radial transfer function, and its dependence on age, MP, heart rate (HR), ejection duration (ED), PP amplification (PPA), brachial PP and aortic augmentation index (AIX). The database [JAHSH 2008;2:20] comprised 8212 observations in 1505.

Radial artery FF was 33.6%, varying from 17.6% to 50.9% with SD 4.4%. Aortic FF was higher (P<0.001) at 41.0%, varying from 25.0% to 60.0%, with SD 4.2%. FF at both sites were similar in males and females. There was a significant (P<0.001) relationship between radial FF and age, MP, HR, ED, PPA and Aix.

Wide variation in radial artery FF results in substantially different values of MP being calculated if a set formula such as diastolic/H11001 FF and age, MP, HR, ED, PPA, PP and AIx. is suitable for use of applanation tonometry to calculate MP in the upper limb.

IS THE PHASE OF THE MENSTRUAL CYCLE IMPORTANT WHEN SCREENING FOR PRIMARY ALDOSTERONISM (PAL) IN WOMEN, AND DOES RENIN ASSAY METHOD MATTER?

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Increased oxidative stress has been suggested to contribute to hypertension. Although omega-3 (-3) fatty acid deficiency has been shown to increase oxidative stress, development of hypertension has been inconsistent. The aim of the present study was to determine whether elimination of the antioxidant selenium (Se) would lead to hypertension in animals maintained on the -3 fatty acid deficient diet. At 7 weeks of age, male Sprague-Dawley rats (N=10) were divided into two groups and placed on semi-synthetic diets that contained identical amounts of protein (15%; from torula yeast 30% w/w), carbohydrate (53% w/w), fat (7% w/w), AIN-93 vitamins and minerals except for Se. The diets were either sufficient (ω3:1:7; 3%; canola oil; α-linolenic acid) or deficient (ω3:1:7; 7% safflower oil; linoleic acid) in ω3 fatty acid, and were either sufficient (Se > 4.2 mg/kg) or deficient (Se < 0.05 mg/kg). In Se-deficient groups, the diet was also deficient in Se (systolic blood pressure ω3:1:7:124:1:10:11 mg/mmHg vs ω3:1:7:136:1:10:9 mg/mmHg, P<0.05; ω3:1:7:124:1:10:11 mmHg vs ω3:1:7:118:11:15 mmHg, ns). In addition, Se deficiency did not alter blood pressure in animals maintained on the ω3 deficient diet. Heart rate, body weight, food and water intake were not different between groups. Thus, it would appear that Se, due to its antioxidant actions, can ameliorate the hypertensive effects of ω3 fatty acid deficiency.

DOES SELENIUM DEFICIENCY EXPOSE THE HYPERTENSIVE EFFECT OF AN OMEGA-3 FATTY ACID-DEFICIENT DIET?

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ACUTE CARDIOVASCULAR RESPONSE TO STRESS: MODULATION BY THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

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Early elevation of blood pressure (BP) is common and predicts haematoma growth and other adverse outcomes in acute intracerebral haemorrhage (ICH). However, uncertainty persists as to whether there are beneficial effects of early BP lowering treatment. We used data from the Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trial (INTERACT) pilot phase to determine the importance of systolic BP levels at presentation and achieved in the first 24 hours, as predictors of absolute and relative haematoma growth in ICH. INTERACT included 404 patients with elevated systolic BP (150 –220 mmHg) within 6 hours of CT-confirmed ICH. Digital images of baseline and repeat CT (24:3 hours) were performed using standardised techniques and analysed in an independent core laboratory. The effect of BP levels on absolute and proportional haematoma growth in haematomas volumes were assessed by an analysis of covariance (ANCOVA) with sex, log of baseline haematoma volume, haematoma location, time from CT to ICH onset, and randomised treatment, as covariates. Overall, 346 patients with 2 CT scans were available for analyses. Absolute growth in haematoma volume increased with higher baseline BP levels: 2.1 ml (95% CI 1.7, 2.5 ml) per mmHg increase in baseline BP level after initiation of such treatment were more important predictors of haematoma growth than baseline BP levels in acute ICH.
ENDOTHelial dysfunction in aged spontaneously hypertensive rats is reversed by NADPH Oxidase Inhibition

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Arterial hypertension is associated with increased formation of reactive oxygen species (ROS). ROS react with NO, reducing the bioavailability of this vasorelaxant. The resultant impaired endothelium-derived vasorelaxation, i.e. endothelial dysfunction is a hallmark of cardiovascular diseases such as hypertension. NADPH oxidases are enzymes that solely produce ROS, and hence represent a novel target for blood pressure reduction via inhibition of ROS formation.

To examine ROS formation and the expression of the different NOX isoforms NOX1, 2, and 4 in aortae of aged spontaneously hypertensive rats, a model of endothelial dysfunction and oxidative stress, ROS formation was measured by dihydroethidium (DHE) staining of tissue sections or by lucigenin chemiluminescence of aortic homogenates. NOX expression was measured by Western blotting and immunofluorescence analysis of NOX proteins by confocal microscopy. In conclusion, ROS formation is increased in aged SHR, a greater reduction of blood pressure and lumbar sympathetic nerve activity compared to WKY rats aortae compared to WKY rat aortae (3.4 ± 0.6, P < 0.01 and 1.6 ± 0.1, P = 0.01, respectively), whereas NOX4 expression remained unchanged. In tissue sections NOX1 showed strong positive staining in the intima of aorta, where it co-localized with an endothelial cell marker. NOX1 staining was only weakly positive in the aortae of WKY. NOX2 distribution was similar in both rat strains. Aortic endothelial function, as indicated by the maximal relaxation response to acetylcholine, was significantly impaired in SHR versus WKY rats (SHR: 56 ± 1.1% versus WKY: 67 ± 2.7%, P = 0.05). The NADPH oxidase inhibitors VAS20270 (10 μM/L) and apocynin (100 μmol/L) improved the relaxation in aortae of both WKY rats (79.4 ± 2.2% and 80.2 ± 2.6%, respectively) and even more pronounced in SHR (80.6 ± 3.6% and 77.8 ± 4.9%, respectively) resulting in similar relaxation of SHR and WKY aortae. In conclusion, ROS formation is increased in aortae of aged SHR and hence represent a novel target for blood pressure reduction via inhibition of ROS formation.
CVD IN INDIGENOUS AUSTRALIANS: OPPORTUNITIES FOR IMPROVING OUTCOMES ACROSS THE CONTINUUM OF CARE

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Whilst recent political and health system reform has focused on ‘closing the gap’ in health status for Indigenous Australians, less attention has been afforded to outlining the specific activities which are most likely to reduce this gap. Amongst a long list of health issues driving the disparity experienced by Indigenous Australians, Cardiovascular Disease (CVD) remains the primary target. They are the principal activities which are most likely to reduce this gap. National data has limited information exists suggests that there are significant barriers to necessary care for quality and outcomes of health system performance in explaining these differentials. What between the ages of 25–54 years.

In terms of explaining these gaps, most attention has focused on the higher burden of traditional risk factors experienced by Indigenous people. Far less attention has focused on the quality and outcomes of health system performance in explaining these differentials. This presentation reports on extensive qualitative and quantitative clinical research of CVD in the NT, focused on the patterns, burdens, provision of care, experience of services, adverse outcomes and their determinants, and opportunities for reform within the management of CVD among Aboriginal Australians.

INCREASED TISSUE KALLIKREIN EXPRESSION IN HUMAN TYPE 2 DIABETES

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The kallikrein kinin system contributes to inflammation and organ-protection. Loss of function mutation of the tissue kallikrein gene is associated with arterial dysfunction in humans and gene knockout studies show an essential role for tissue kallikrein in arterial function, ischemic preconditioning, cardiac remodeling and survival after myocardial infarction. Moreover, kinin peptides mediate in part the benefits of angiotensin converting enzyme inhibitor and angiotensin II type 1 receptor blockers therapies. To investigate the expression of the kallikrein kinin system in human type 2 diabetes mellitus we measured circulating levels of bradykinin and kallidin peptides, high and low molecular weight kininogens, plasma and tissue kallikrein, and kallistatin in non-diabetic and diabetic patients before coronary artery bypass graft surgery. Plasma levels of tissue kallikrein were approximately 62% higher in diabetic than non-diabetic patients (P = 0.015), whereas there were no differences in circulating levels of bradykinin and kallidin peptides, high and low molecular weight kininogens, plasma and tissue kallikrein, and kallistatin in non-diabetic and diabetic patients before coronary artery bypass graft surgery. In conclusion, our preliminary array data has identified novel genes whose hypothalamic expression is altered in hypertension. Some of these may be causative and others include ones changing to maintain or counteract the hypertensive state.

COMPARISON OF VARIOUS ANTHROPOMETRIC INDICES AS PREDICTORS OF CARDIOVASCULAR DISEASE IN PATIENTS WITH TYPE 2 DIABETES

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Aims: The aim of this study was to compare the strength of associations and discrimination capability of body mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR) with the risk of cardiovascular disease and death in patients with type-2 diabetes participating in ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation).

Methods and results: 11,140 men and women were followed for a mean of 4.8 years. Cox proportional hazard models were used to determine the hazard ratios (HR) and 95% confidence intervals (95% CI) for the risk of cardiovascular disease and death, associated with one standard deviation (SD) increase in baseline BMI, WC and WHR. After adjustment, HR (95% CI) for WC were 1.10 (1.03–1.18) for cardiovascular events, 1.13 (1.03–1.24) for coronary events, and 1.09 (0.98–1.19) for cardiovascular deaths. Estimates for WHR were 1.12 (1.05–1.19), 1.17 (1.08–1.28) and 1.19 (1.09–1.31). BMI was not significantly associated with the risk of any of these outcomes. While the receiver operating characteristics curve could not differentiate between the predictive capacity of these anthropometric variables (p-values = 0.295), a discriminative improvement statistic showed an enhancement in the discrimination capabilities of models using WHR for cardiovascular outcomes, except for cerebrovascular events.

DID YOU KNOW

The amygdala located in the limbic system has long been associated with mediating the emotional and hormonal response to stress. While there has been much focus on the role of the central nucleus of the amygdala (CeM), there is an increasing awareness that the medial nucleus of the amygdala (MeA) is a major region activated to specific “stressful” stimuli. Studies in rats have shown that the MeA was the only region of the amygdala to increase in BP during the change from lights on to off was only associated with a greater activation, as detected by c-Fos expression, in the MeA (9.9 ± 0.9/mmHg, P < 0.001) and paraventricular nucleus (30%; P < 0.05) of the hypothalamus. However, the greater increase in BP during the change from lights on to off was only associated with a greater activation, as detected by c-Fos expression, in the MeA (9.9 ± 0.9/mmHg, P < 0.001) and paraventricular nucleus (30%; P < 0.05) of the hypothalamus. In conclusion, our preliminary data has shown that the MeA is the only region of the amygdala to respond to a cat smell suggesting it is involved in the integration of threatening olfactory stimuli. In our studies of the Schlaer hypertensive (BP/H2J) mouse, we found that the pressor response to cage-swap stress was markedly greater compared with the normotensive (BP/H3J) mice (1c–1.2 vs 3±1.1 mmHg, P < 0.001). Elevated BP was also associated with greater activation of the MeA (+63%, P < 0.05) and CeM (+35%, P < 0.001) compared with the mosodermal (+40%, P < 0.001) and paraventricular nucleus (+30%; P < 0.05) of the hypothalamus. Moreover, the greater increase in BP during the change from lights on to off was only associated with a greater activation, as detected by c-Fos expression, in the MeA (9.9 ± 1.1 vs 3±1.1 mmHg, P < 0.001) and not the CeM. Indeed the absolute level of BP in the normotensive and hypertensive animals during the night and day periods very closely correlated (r=0.98) the degree of activation observed in the MeA. The latter was also closely correlated with the effect of pentolinium to lower BP via inhibition of the sympathetic nervous system (r = 0.96). In both cases, the correlation was stronger than for any other region examined. These studies suggest that the MeA, normally activated by specific fear inducing stimuli may inappropriately respond to the normal arousal
associated with night-time active periods in the Schlager (BPH/2J) hypertensive mice leading to hypertension and an exaggerated circadian day-night difference in BP.

TOPOGRAPHY AND PROJECTION OF BLOOD PRESSURE SENSITIVE NEURONS IN THE LATERAL PARABRACHIAL NUCLEUS

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The lateral parabrachial nucleus (LPBN) is increasingly viewed as a critical integrative site for the transfer of visceral cardiovascular information from the brainstem to a number of regions in the forebrain. The goals of this study were to examine the topography and relationship between BP sensitive neurons located in LPB subnuclei and their projections to the median preoptic nucleus (MnPO) and central nucleus of the amygdala (CeAm). Retrogradely transported neuronal tracer was injected into either the MnPO (n=8) or the CeAm (n=8) and rats underwent a 1-week recovery period. C-Fos immunohistochemistry was used to detect neurons in the LPB activated in response to iv infusion of phenylephrine hydrochloride (10 µg/kg/min; n=8) or sc injection of diazoxide (45 mg/kg; n=8) in experimental groups; or iv infusion or sc injection of isotonic saline (n=8) in control groups. Neuronal activation in rats infused with iv phenylephrine was greater in the central dorsal, central part of the external, and superior lateral PB subnuclei (P<0.001) compared with vehicle infused controls. The findings demonstrate that effector projections arising from neurons in the central (43%), dorsal (31%) and dorsal part of the external (60%) lateral PB subnuclei innervating the MnPO also respond to an increase in BP. Alternatively, 63% of neurons located in the central part of the external lateral FN that innervate the CeAm were sensitive to a rise in BP, although neuronal activation in rats with reduced arterial pressure induced by sc injections of diazoxide was greater in the central LB and central part of the external LPB subnuclei compared with saline infused controls, these cells were not retrogradely labelled from either the MnPO or the CeAm. This is the first demonstration of direct neural projections arising from pressor responsive neurons in discrete LPB subnuclei innervating neurons in the MnPO and CeAm. Although, there was no evidence for ascending projections from neurons activated by hypotension terminating in either the MnPO or CeAm, this study does provide topographic evidence for separate populations of neurons in the LPB responding to elevated and reduced BP.

EXPRESSION OF RENALASE, A NOVEL MONOAMINE OXIDASE, IN ADIPOSE TISSUE

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The prevalence of obesity is increasingly recognised as important risk factors for the development of a number of cardiovascular-related conditions including hypertension. The precise mechanisms linking obesity to hypertension are still only incompletely understood. One of the mechanisms governing this increased risk involves activation of the sympathetic nervous system. Up till recently membrane bound monoamine oxidases (MAO A/B) and semicarbazide-sensitive amine oxidase have been responsible for the breakdown of catecholamines in human tissues and in circulation. Renalase, a novel soluble monoamine oxidase, has been identified in the human kidney and heart. Given that adipose MAO A/B expression and activity in obesity is altered, in the present study we aimed to determine whether renalase is synthesised by adipocytes and whether its expression is altered with weight loss in patients with the metabolic syndrome.

Twenty-three subjects with metabolic syndrome participated in this study. To assess the effects of weight loss on sympathetic activity they were randomised into a diet, diet and exercise or control (no treatment) group. Indices of sympathetic activity included whole body noradrenaline spillover and muscle sympathetic nerve activity and were measured at baseline and after 3 months of treatment. Abdominal subcutaneous fat biopsies were also obtained at the same time points. Soluble protein extracts from adipose tissue were separated by SDS-PAGE and analysed by western blotting to detect renalase.

As previously reported, weight loss was associated with a significant reduction in both whole body and muscle sympathetic nerve activity. Renalase was readily detectable in all adipose tissue samples obtained. In control subjects no change in renalase abundance was detected. However, the abundance of renalase was reduced in patients who achieved weight loss with diet, when compared relative to the loading marker protein, GAPDH. Furthermore, we report renalase expression in adipocytes and preadipocytes.

The novel soluble monoamine oxidase renalase is expressed in subcutaneous adipose. Diet induced weight loss reduces both sympathetic activity and renalase expression in adipose tissue. These findings indicate that renalase may be involved in sympathetic regulation in patients with the metabolic syndrome.

HYPERLEPTINEMIA CONTRIBUTES TO HYPERTENSION BY SYMPATHOEXITATION IN OBESITY

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Leptin is a hormone released from fat tissue which promotes weight loss by reducing appetite and by increasing energy expenditure through activation of the sympathetic outflow to thermogenic tissues. In obese states there is a resistance to the metabolic (satiation and weight-reducing) actions of leptin. Leptin resistance appears to be selective, and it is possible that leptin activates some neurons that regulate the sympathetic nervous system (SNS) in obesity. To evaluate the hypothesis that stimulation of SNS by leptin causes an increase in intercapillary temperature (IT), blood pressure (BP), and heart rate (HR), mice were fed a high fat diet (45 % fat), Diet induced obese (DIO) mice were compared with mice on a control diet (12% fat) and with genetically obese leptin deficient (ob/ob) mice. Sympathetic outflow to brown adipose tissue (BAT), and locomotor activity was measured. Although SNS was increased in DIO mice compared with control mice (4.9 C, P<0.001). On the contrary, ob/ob mice showed lower IT than controls (P<0.001). LA was similar in DIO and controls, but ob/ob mice were hypothermic. DIO mice had a significant increase in IT after peripheral or central leptin administration, despite no difference in caloric intake, showing that the thermogenic response to leptin was intact. Leptin also induced p-Stat3 expression (a well-known mediator of leptin activation) in dorsal meddial (DMH) but not in arcuate nucleus of the hypothalamus of DIO mice. Leptin given directly into the DMH significantly increased IT in all groups. Mean arterial pressure (MAP), measured by radiodensitometry, was higher in DIO mice than lean controls (115±1.7 vs 103±1.5 mmHg, P<0.001) as well as systolic BP (128±1.2 vs 117±1.5, P<0.01), diastolic BP (100.7±1.9 vs 89.3±1.3, P<0.001) and HR (550.4±11.5 vs 475.9±7.2, P<0.001). Chronic peripheral leptin infusion increased systolic BP in lean but DIO mice (P<0.05). Leptin levels strongly correlated with MAP (r=0.71) and IT (r=0.72, P<0.01). These results indicate that leptin is resistant to leptin’s suppressive effect on appetite, but not to the sympathetic regulation of BP and BAT. This strongly suggests that DHA may mediate the sympathetic responses during hyperleptinemia.

FLOW AND ENERGY LOSS IN SACCUAR ANEURYSMS ARISING FROM STRAIGHT AND CURVED PARENT ARTERIES - HAEOMODYNAMIC ANALYSIS FOR ASSESSING RISK OF RUPUTE

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Saccular aneurysms are the most common type of cerebral aneurysms with 50% mortality due to rupture. Although risk of rupture is conventionally associated with aneurysm size, development of imaging technology and computational fluid dynamic (CFD) analysis has enhanced the scope of investigations of rupture beyond aneurysm size and shape. The aim of this study was to investigate the effect of parent artery geometry on intra-aneurysm flow patterns and energy loss (EL). CFD analysis was performed on finite volume models of saccular aneurysms fed with curved or straight parent arteries ranging from 4, 5 and 6 mm diameter; aneurysm depth 6–10 mm; aneurysm neck width 4.8 mm. Calculations were performed with pulsatile and steady flow rate (300 ml/min) at inlet and outlet. EL was calculated as the energy difference between arterial inlet and outlet values for each parent artery diameter of straight and curved parent artery models. The result indicated that EL was not dependent on aspect ratio (depth/neck width) over the range 1.25–2.1. However, appearance of secondary flow in aneurysms fed by curved parent arteries increased the magnitude of EL two fold compared to those fed by straight parent arteries. A small (4 mm) curved parent artery resulted in the highest relative EL and flow rate inside the aneurysm for all aspect ratios. In conclusion CFD analysis shows that intra-aneurysm flow patterns and energy loss due to the saccular malformation are affected by the geometry of parent arteries. Since flow changes can affect both growth and rupture of aneurysms, this analysis can be used to differentiate aneurysms of similar size but with different risk of rupture.

HYDROGEN SULPHIDE INHIBITS PLATELET DENSE GRANULE SECRETION BUT NOT PLATELET ACTIVATION

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Hydrogen sulphide is a gaseous mediator endogenously generated in the heart and blood vessels. It has been shown to cause vasorelaxation and plays a role in cardioprotection from ischemic injury. Recent studies have shown that extra-physiological but sub-toxic doses of hydrogen sulphide may inhibit platelet aggregation in vitro. We sought to elucidate the mechanism by which such inhibition might occur. Platelet rich plasma was obtained from six healthy volunteers and incubated with 10 µM hydrogen sulphide or pH matched vehicle controls for 20 seconds prior to initiation of platelet activation and aggregation with chemical agonists. While samples treated with hydrogen sulphide exhibited normal shape change and initiation primary aggregation, secondary platelet aggregation was inhibited by hydrogen sulphide versus vehicle control in response to 0.5 µM arachidonic acid (5.6% vs 81.4%, P<0.001), 2 µM collagen (28% vs 82%, P<0.01), 0.5 µM of thromboxane mimetic U46619 (38.4% vs 67.7%, P<0.01) and 7 µM adrenaline (19.6% vs 76.5%, P<0.001). In hydrogen sulphide treated blood, marked disaggregation was observed following normal initial shape change and primary aggregation in response to 2 µM adenosine diphosphate (20.2% vs 72.7%, P<0.01) indicating that while platelet activation was normal, stability of the platelet aggregates was disturbed by hydrogen sulphide. ATP release measured by luciferase was completely inhibited by hydrogen sulphide for all chemical agonists tested, while P-selectin expression, a novel solu flow cytometry, was unaffected and the structure and function of the platelet fibrogen receptor GPIIb-IIIa, as reported by PAC1 binding, was also unaffected. This suggests that hydrogen sulphide inhibits platelet contraction and secondary aggregation leading to reduced stability of platelet aggregates through prevention of dense granule release and therefore the secretion of important signalling molecules such as ATP, ADP, adenosine, ionic calcium and serotonin. However, primary platelet activation, initial aggregation, shape change and release of alpha granules containing adhesion molecules such as P-selectin are unaffected. Therefore hydrogen sulphide may have significant therapeutic benefit by preventing propagation of secondary platelet aggregation without altering primary platelet activation and aggregation in response to injury and exposure to sub-endothelial collagen, thus potentially ameliorating the increased bleeding risk associated with other forms of antiplateterapy.
HOMOCYSTEINE AND DNA DAMAGE GENETIC POLYMORPHISMS ARE ASSOCIATED WITH HYPERTENSION IN PREGNANCY

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Hypertensive disorders of pregnancy are a major cause of both maternal and fetal morbidity and mortality. Preeclampsia affects 5–8% of all pregnancies, while 10–20% of mothers will have a hypertensive disorder during pregnancy. Previous studies have associated the C677T mutation in methylenetetrahydrofolate reductase (MTHFR) gene polymorphism and plasma homocysteine concentration with hypertension, preeclampsia and DNA damage. This was a prospective study involving 1169 nulliparous pregnant couples. Samples and patient information were collected by SCOPE research midwives. Genotyping was performed by Sequenom MassARRAY. Non-European couples and those who required fertility treatment were excluded from analyses. Pregnancy outcomes were strictly classified: PE (n = 71), PE + SGA (n = 20), controls (n = 408). Chi Square and univariate ANOVA with post-hoc analyses were performed. Both paternal and neonatal methionine synthase (MTHFR) 2756 G alleles were associated with PE + SGA compared to controls (P = 0.033 resp., P = 0.043 respectively). Paternal methionine synthase reductase (MTRR) GG genotype was associated with PE + SGA compared to healthy pregnancies (P = 0.030). Neonatal NAT1*10 allele connected for age and BMI was associated with maternal mean arterial pressure and sBP at 15 weeks gestation. These findings are in agreement with clinical data whereby vitamin D deficiency is linked to heart disease.

ASSOCIATION OF CARDIAC FUNCTION WITH TRANS-THORACIC ECHOCARDIOGRAPHY IN ADULT VITAMIN D DEFICIENT RATS

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Vitamin D is a fat-soluble vitamin which is essential in bone metabolism, cell growth, differentiation and regulation of the immune system. The amount of vitamin D received from the skin to sunlight is the major source of vitamin D. There is an increasing prevalence of vitamin D deficiency in many populations world-wide, resulting from both inadequate exposure to ultraviolet light and diet intake. It is well known that vitamin D deficiency is associated with heart disease. We have recently demonstrated in the rat heart that vitamin D deficiency leads to cardiac hypertrophy and vulnerability to ischemia later in life, and female offspring appear to be most vulnerable. The aim of the present study was to investigate the effect of vitamin D deficiency on cardiac function, using echocardiography, in 14 week old adult rats. Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. At weaning the offspring remained on their respective diets until adulthood. At 14 weeks of age non-invasive trans-thoracic echocardiography was performed in female offspring (n = 10 control and n = 9 vitamin D deficient). M-mode echocardiographic images were acquired in the midsystolic phase of the left ventricle. Anterior, posterior end-diastolic and end-systolic wall thickness, left ventricular internal dimensions and interventricular septum were measured. Body weight was not different in control and vitamin D deficient offspring at 14 weeks of age. Left ventricular weight and left ventricular weight to body weight ratio was significantly increased (P < 0.001) in vitamin D deficient offspring. This was accompanied by a significant decrease in diastolic volume (P = 0.0002) but no difference in systolic volume. In addition, stroke volume and cardiac output in the vitamin D deficient offspring were significantly reduced (P < 0.001). Fractional shortening and ejection fraction were unaltered in both control and vitamin D deficient female offspring. In conclusion, long term vitamin D deficiency in female rats leads to left ventricular hypertrophy and impaired cardiac function. The findings are in agreement with clinical data whereby vitamin D deficiency is linked to heart disease.

EFFECT OF VITAMIN D DEFICIENCY ON CARDIAC FUNCTION AND SUSCEPTIBILITY TO ISCHEMIA/REPERFUSION INJURY IN THE ADULT RAT HEART

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Exposure to vitamin D deficiency in utero and early life leads to delayed maturation and subsequent enhanced growth (proliferation and hypertrophy) of cardiomyocytes in the left ventricle. The implications of these changes on cardiac function later in life are unknown. The aim of the present study was to investigate the effect of vitamin D deficiency in adult rats on cardiac function and the susceptibility to ischemia/reperfusion injury. Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. Offspring remained on their respective diets until adulthood. Hearts of 16 week old male and female offspring (n = 8/group) were mounted on a Langendorff apparatus. Basal heart rate (HHR), coronary flow, rate of contraction (+ dp/dt) and relaxation (- dp/dt) and response to isoprenaline were recorded. The hearts were then subjected to 20 minutes ischemia and 1 hour reperfusion. At the end of the reperfusion period the left ventricle was sliced and incubated in 1% 2, 3, 5 triphenyl tetrazolium solution (T2T), to determine infarct area using computerized planimetry. Basal cardiac function (HHR, + dp/dt, - dp/dt) was not different between groups. Basal coronary flow was lower in hearts of vitamin D deficient rats. The isoprenaline-induced increase in HR tended to be greater in vitamin D deficient males (P = 0.06), but there was no differences in contractile function between groups. After 55 minutes reperfusion, HR had declined by 30% of that before ischemia in both males and females, with HR being higher in vitamin D deficient males compared with control males. Infarct area was 2-fold greater in vitamin D deficient hearts of both males and females (P = 0.006 & P = 0.03, respectively). Basal and stimulated heart function was not altered, although coronary flow was significantly reduced in vitamin D deficient rats. In conclusion, the hearts of vitamin D deficient rats are particularly susceptible to ischemia/reperfusion injury. Dysregulation of coronary flow and the extent of vascularisation may be factors which contribute to the increased susceptibility to ischemia/reperfusion injury.

THE THIAZIDE-SENSITIVE NA-CL COTRANSPORTER IN THE DISTAL NEPHRON (NCT) IS REGULATED BY BOTH KINASES AND PHOSPHATASES

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Dissecting the molecular regulation of renal sodium transport by the thiazide-sensitive NCT has provided important insights into the mechanisms underlying hypertension. NCT regulation is complex involving a scaffold, MPR-A, which includes SPAK and WNK4 kinases. SPK kinase activity is the key regulator of NCT activity and although WNK4 kinase activity is known to be modulated by other kinases, the in vivo role of SPK and WNK4 remains to be defined. In this study we propose a model to characterize the pressure distribution between the intra and extra ocular space. Electrical circuit elements are used to construct the model. Therefore in this study we propose a model to characterize the pressure distribution between the intra and extra ocular space. Electrical circuit elements are used to construct the model. The retinal venous pressure is obtained as a function of the difference between IOP and CSF. Simulation results show an increase in CSF outflow resistance will increase the CSF compliance. The retinal venous pressure is obtained as a function of the difference between IOP and CSF. Simulation results show an increase in CSF outflow resistance will increase the CSF compliance.
**NEUROPEPTIDE CODING OF ADRENALECTOMY SYMPTOMATIC PREGANGLIONIC NEURONS**

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Different physiological stimuli selectively evoke release of noradrenaline (NAO) or adrenaline (Ad) from the adrenal medulla. Therefore NAO and Ad chromaffin cells are innervated by different populations of sympathetic preganglionic neurons (SPN). How these populations differ functionally is not completely understood. It has been suggested that 30% of SPN innervating the adrenal medulla are chemically coded. The neuropeptides pituitary adenylate cyclase activating polypeptide (PACAP) and enkephalin have been proposed as neuromodulators mediating catecholamine secretion from the adrenal medulla based on immunohistochemical localization of terminal adrenal neuronal chromaffin cells. The aim of this study was 1) to determine the distribution and proportion of SPN that contain PACAP or preproenkephalin (PPE) mRNA, 2) to determine the distribution and proportion of adrenergically projecting SPN that contain PACAP or PPE mRNA and, 3) in light of the proposed sensory innervation of the adrenal medulla, to determine whether sensory neurons in the dorsal root ganglion (DRG) that innervate the adrenal medulla contain PACAP or PPE mRNA. Digoxigenin-labeled riboprobes encoding PACAP and PPE were synthesized. Following anesthesia and perfusion, spinal cord sections from male Sprague-Dawley rats were processed for in situ hybridization (ISH) for PACAP and PPE combined with immunohistochemistry (IHC). Study 1: PACAP/PPE ISH combined with IHC for vesicular acetylcholine transporter (vAChT). Study 2: PACAP/PPE ISH combined with IHC for cholina receptor B (CTB–B, identifying adrenergically projecting neurons after CTB injections into the adrenal medulla). We found in total SPN that few were PPE+ (4.2%) whilst the majority were PACAP+ (80.3%). In adrenergically projecting SPN, 47.3% were PPE+, whilst 97.3% were PACAP+. In adrenergically projecting DRG 1.1% were PPE+, whilst 74.4% were PACAP+. In summary, we found that the majority of total SPN contain PACAP mRNA while few SPN contain PPE mRNA. We have established that all adrenergically projecting SPN contain PACAP and 50% of preproenkephalin containing SPN contain enkephalin. This study implies that the adrenal medulla receives sensory innervation that is largely PACAPergic. This may provide a neurochemical basis for differential control of LC versus 50% are also enkephalinergic. Also, the adrenal medulla receives sensory innervation that is not be restricted to renal and cardiac tissues. Human tissue samples were obtained from the Victorian Institute of Forensic Medicine (VIFM) Tissue Donor Bank at autopsy. The informed consent of the donor’s next-of-kin was obtained before the autopsy. All protocols were approved by the VFM Ethics review Committee. Total RNA and soluble protein were extracted from these tissues. RT-PCR and western blotting were performed to determine the evidence of renalseque gene expression. Our results demonstrate that renalseque has a larger representation in human tissues than previously reported and is not restricted to the kidneys and heart. Western blotting showed the expression of renalseque in a variety of regions of the central nervous system, peripheral nervous systems, in adrenal glands, adipose tissue, and peripheral blood mononuclear cells. RT-PCR was used to validate these observations and enable the identification of several splice variants of the renalseque transcript. These splice variants appear to be tissue specific and point to a role of renalseque function in the structural and functional characteristics of renalseque may help to facilitate our ability to diagnose and treat disorders involving an imbalance in the levels of mononuclear neurotransmitters. We are currently pursuing investigations into the regulation of renalseque gene expression using a variety of cell culture and animal models.

**INCREASED NO-DEPENDENT DILATION OF RAT ARTERIOLES IN DIET-INDUCED OBESITY**

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Obesity is an established risk factor for hypertension and associated cardiovascular disease and impairs blood vessel function. The large-conductance Ca2+-activated potassium channel (BKCa) has a critical role in the control of arterial diameter and may play a key role in the pathogenesis of hypertension. We have previously reported BKCa function in rat skeletal muscle arteries is impaired in diet-induced obesity. The aim of this study was to examine the effect of diet-induced obesity on endothelium- and BKCa-dependent dilation of rat skeletal muscle arteries. Male Sprague-Dawley rats (7–8 weeks) were fed a cafeteria-style high fat or control diet for 16 weeks. Following anesthesia with sodium thiopentone (100 mg/kg i.p.), studies were performed in isolated, pressurized (70 mmHg) first-order arterioles from the cremaster muscle. Control rats weighed 570±7 g compared with obese rats 768±13 g (n=35–37 of each, P<0.05). Electron microscopy of cremaster muscle arterioles showed remodeling associated with both media thickness and lumen to media ratio significantly increased in diet-induced obese rats (n=10 of each). Despite this, diet-induced obesity had no effect on acetylcholine (Ach, 0.001–3 μM)-induced dilation of arterioles. The NOS and guanylate cyclase (GC) inhibitors L-NAME (100 μM) and ODQ (10 μM) did not alter myogenic tone in arteries from control rats, however, in arterioles from obese rats diameter was significantly reduced (observed baseline 45.5±1.2%; L-NAME/ODQ 38.1±1.6%, n=11, P<0.05). Ach-induced dilation of arterioles from control rats was completely abolished by a combination of the BKCa blockers TRAM-34 (0.1 μM) and apamin (0.1 μM) and barbiturate (BHT, 0.1 μM), respectively, with no apparent role for NOS as assessed by the lack of effect of L-NAME/ODQ (n=10–15 of each). In arterioles from obese rats however, BHT had no effect on responses to Ach while the NO/GC inhibitors partially inhibited ACh-induced dilation (maximum, 3 μM Ach, 97.2±2.1% vs maximum L-NAME/ODQ 82.6±2.7%, n=12–15 of each, P<0.05). Western blotting showed decreased expression of the BKCa subunit in arterioles from obese rats. Expression of eNOS was not altered by obesity, however there was an accumulation of complexed forms of caveolin-1 and -2 at cemaster arteriole membranes. In summary, diet-induced obesity resulted in an alteration in the relative contribution of NOS and BKCa to Ach-mediated endothelium-dependent dilation of rat cemaster muscle arteries. BKCa-dependent dilation was abolished in obesity while an NO-component was established.

**ASSOCIATION OF HYPOPHYSALITARY PITUITARY AXIS GENES WITH LONGITUDINAL CHILDHOOD SYSTOLIC BLOOD PRESSURE**

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Childhood blood pressure is predictive of adult hypertension. Genes of the hypophyseal pituitary axis may affect blood pressure control. In adult studies, association studies have shown that the NRR3C variant is associated with adult obesity and hypertension. The aim of the study was to identify genes which are related to longitudinal blood pressure in childhood. The Raine Pregnancy Cohort (n=2009) has recorded oculometric resting blood pressure on children at age 1, 2, 3, 4, 5, 6, 8, 10 and 14 years. Linear mixed effects models were used to identify trajectories for each sex. Forty-three SNPs were surveyed and 10 hypophyseal pituitary axis genes were selected and tagged to ensure complete coverage of the genes taking into account linkage disequilibrium blocks. The single nucleotide polymorphisms (SNPs) from the list of genes were analysed by multivariate linear modelling for association with systolic blood pressure intercept and trajectory separately for boys and girls. Of the 44 HPA axis genes, 44 SNPs were scored. 44 SNPs were associated with systolic blood pressure in females and 42 SNPs genes of 15 genes were associated with blood pressure in males (all P<0.05). The following SNP’s were associated with blood pressure trajectories or intercepts: 15 SNPs of nuclear receptor subfamily 3 (NRR3C), 12 SNPs of insulin growth factor 1 receptor (IGF1R), 6 SNPs of insulin receptor growth factor 2 receptor (IGF2R), 9 SNPs of (proprotein convertase subtilisin) PCSK2, 5 SNPs of peroxisome proliferator-activated receptor gamma (PPARγ2), 5 SNPs of leptin and 5 SNPs of leptin receptor. Increased BP trajectories in adolescence was associated with SNPs in 10 genes in males, 13 genes in females and 6 genes common to both sexes (RS1T, IGFC, LEP, NR3C2, PPARG2, PCSK2). Conversely, decreased BP in adolescence was associated with SNPs in 11 genes in males, 10 genes in females and 3 genes common to both sexes (GHRL, NR3C2, PCSK2). In conclusion, this study suggests that the
genes of the hypothalamic axis, in particular NRC2, IGFR1, IGFR2, PCSK2, leptin, leptin receptor and PPARα may have a role in childhood blood pressure control. These results are currently being replicated in other pregnancy cohorts.

A CROSS-SECTIONAL STUDY ASSESSING THE RELATIONSHIP BETWEEN DIETARY SODIUM AND BLOOD PRESSURE IN AN AUSTRALIAN POPULATION SAMPLE

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Excess sodium consumed throughout life contributes to the age-related rise in blood pressure (BP). Reducing dietary sodium or the dietary sodium to potassium ratio lowers BP. The relationship between dietary sodium and potassium intake and BP within an Australian population group has not previously been assessed. The aim of this study was to assess the relationship between dietary sodium and potassium intake and blood pressure in an Australian population sample, using the gold standard measurement of 24 hr urine excretion. A cross-sectional study was conducted using participants enrolled in the Melbourne Collaborative Cohort study. Daily intakes of sodium and potassium were measured from 24 hr urine samples provided by participants (n=706). BP was assessed under standard conditions in a subgroup of this population. The mean age of participants (men n=376, women n=408) was 64.0 (6.3) (SD) years. For men and women respectively, the mean urinary sodium was 178.4 (66.6) and 133.7 (51) mmol/day (approximately 10 g and 8 g salt/day), mean urinary potassium was 88.1 (30.7) and 77.0 (23.9) mmol/day and the sodium to potassium (Na/K) ratio was 2.16 (0.88) and 1.93 (0.74) mmol/day. Only 21% of participants met the recommended intake of sodium (<100 mmol/day). In the 584 participants who provided blood pressure measurements, sodium and the Na:K ratio remained significant predictors of BP. These results indicate that a population wide reduction in dietary sodium would be effective in reducing blood pressure in Australia.

SYMPATHETIC EXCITATION CAUSED BY PACAP-38 IN THE SPINAL CORD IS MEDIATED VIA THE PAC1 RECEPTOR

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Pulmonary adenylyl cyclase activating polypeptide (PACAP) is an excitatory neuropeptide with known central and peripheral cardiovascular effects. Intrathecal (IT) administration of PACAP increases splanchic sympathetic nerve activity (sSNNA) and HR, but surprisingly elicits no change in MAP. Additionally, it is unknown if PACAP is involved in either the tonic or reflex control of blood pressure, at the level of the spinal cord. Moreover, no pharmacological studies have been performed which of the three PACAP receptors (PAC1, VPAC1 and VPAC2) are responsible for the actions of PACAP at the spinal cord. This study sought to determine if 1) PACAP has a role in the tonic control of blood pressure at the level of the spinal cord, and 2) to determine pharmacologically if the PACAP effects previously reported are mediated via the PAC1 receptor. Urinary adrenaline (3h/kg), in adult male Sprague-Dawley rats (n=12) were vagotomised, ventilated and paralysed. MAP, HR (derived from ECG) and end tidal CO2 were monitored, and sSNNA was recorded. A catheter was inserted into the IT space and advanced to the level of Th6. In n=12 animals, an initial control injection of 10 μl of 10 mM PBS was given. In n=6 of the animals, 10 μl of 1 mM PACAP6–38 (a specific PAC1 receptor antagonist) was injected. Responses were recorded for 30 mins. In the remaining animals (n=6), pretreatment with PACAP6–38 was given 15 mins before 10 μl of 1 mM PACAP-38 (a non-specific agonist) was administered and responses were recorded for 60 mins. PACAP6–38 alone caused no significant change in MAP (7.5±3.3 mmHg), HR (5±2.8 bpm) or sSNNA (10.7±5 %). Following pretreatment with PACAP6–38, PACAP-38 caused significant changes in MAP (20±7 mmHg), HR (17.4±4 bpm) and sSNNA (32±14 %). The HR and sSNNA responses to PACAP-38, following pretreatment with PACAP6–38, were significantly attenuated compared with previous reports of PACAP-38 effects. We conclude that in the urthane anaesthetised, vagotomised, adult male Sprague-Dawley rat, endogenous PACAP is not tonically released onto receptors at the level of the spinal cord. The attenuation of the PACAP-38 response observed following pretreatment with PACAP6–38 indicates that the changes in sSNNA and HR elicited by IT PACAP are mediated primarily via the PAC1 receptor.

CHARACTERISTICS OF EXAGGERATED CARDIOVASCULAR CIRCADIAN RHYTHM IN SCHLAGER HYPERTENSIVE MICE

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The morning surge in blood pressure (BP) is greater and rises faster in hypertensive patients at a time when there is a greater incidence of cardiovascular events. An underlying mechanism may be the exaggerated activation of the sympathetic nervous system. We have recently shown that the BPH (BP high) Schlagier mice are hypertensive due to an overactive sympathetic nervous system and have a greater day night differences in BP and heart rate compared to the BPH (BP normal). The aim of this study was to analyse the characteristics of the surge in BP during the change from inactive to active periods in Schlagier BPH mice. Radio-telemetry devices were implanted in 11 normotensive and 10 hypertensive mice and after 10 days recording, 24 hrs of continuous monitoring was performed. A robust logistic algorithm was fitted to the individual data sets to estimate day and night plateau as well as the rate of change in variables during dark light cycle. The diurnal range in BP and heart rate were 133±4 and 68±6 greater respectively in BPH compared to BPH normotensive mice (P<0.001). However, the rate of rise in BP from inactive to active period is considerably different between the two strains (8.7±2.0 and 8.4±1.9 mmHg/hr). Thus the duration of the surge in BP was markedly longer in the BPH hypertensive animals (3.2±0.5 vs 1.6±0.4 hr, P<0.02) but the midpoint of the rise was similar. Comparison with another normotensive mouse strain (CS7Bl6) showed a similar duration of BP surge (1.7±0.4 hr) compared to the Schlagier BPH normotensive mice. These studies show that during the active period the hypertension is driven by a longer period of sustained activation of the BP surge which starts earlier and finishes later than in BP normotensive counterparts. The mechanisms that regulate the timing of these changes may be key to the understanding the cause of the hypertension in these mice.

CARDIAC AND ANTIOXIDANT EFFECTS OF NOVEL ANGIOTENSIN II RECEPTOR ANTAGONISTS IN VITRO

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A dual action drug containing both AT1 receptor antagonist and antioxidant properties would be a potential therapeutic for the disease atherosclerosis as it comprises of hypertension, inflammation and oxidative stress. The AT1 receptor antagonist milfasartan has been combined with a known potent pharmacophore (selenium, phenol and ebselen) with the aim that they will have dual actions. The AT1 receptor antagonist potency of these compounds has been previously confirmed, with all of the antioxidant-substituted milfasartan analogues, except ebselen-milfasartan, showing antagonist properties in rat isolated right atria. The present study compared the in vitro antioxidant properties in cell and tissue-based assays. The antioxidant properties of the compounds were examined in an AAPH (2,2-azobis (2-amidinopropane) hydrochloride)-induced haemolysis assay (mouse C57/B16 isolated red blood cells) in the presence and absence of 1 mM glutathione (GSH). AAPH results in free radical-mediated haemolysis which is quantified by calculating the area under the absorbance curve. In the absence of GSH neither the antioxidant groups nor milfasartan analogues protected against haemolysis. However in the presence of GSH, ebselen, selenocystein, phenol and phenol-substituted milfasartan protected against lysis (N=4 each; P<0.05 vs. vehicle), whereas the other milfasartan analogues did not. For comparison, the antioxidant capacity of these compounds was also tested in a tissue-based preparation (mouse C57/B16 isolated packed left atria) using doxorubicin (30 μM) as the free radical generator. Doxorubicin resulted in a percentage decrease in left atrial force of 65% over 90 min which can be concentration-dependently reversed by pre-incubating with the antioxidant quercetin (10, 30 and 100 μM). In this assay ebselen and phenol but not selenocystein (10 μM) each protected against doxorubicin-induced negative inotropy. The substituted milfasartan analogues did not protect against oxidative damage, however when pre-treating with milfasartan, phenol-milfasartan was able to protect against radical-mediated damage (N=4 each; P<0.05 vs. vehicle). The data from these experiments showed ebselen-substituted milfasartan is longer acting than phenol-substituted milfasartan, showing antagonist properties that ebselen displays nor the antagonist potency milfasartan possesses. In contrast, the phenol-substituted milfasartan retains AT1 receptor antagonist potency but not antioxidant properties in the tissue-based assay as receptor binding interferes with the antioxidant activity. Although the structure of this interaction may limit this interaction may result in a dual action drug with therapeutic potential for the disease atherosclerosis.

DYNAMIC SYNCHROTRON IMAGING OF DIABETIC RAT CORONARY MICROCIRCULATION IN VIVO

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Endothelial dysfunction in the diabetic coronary circulation plays an important role in the consequent decline in heart function. Conventional angiography is not adequate to assess impairments in micro-vascular function. Using synchrotron imaging we are now able to detect small vessel calibres (≤40 μm, vs. 200 μm using a conventional X-ray device) and quantify regional differences in calibre under conditions of high heart rate (>500 bpm). Experiments were conducted at the Japanese Synchrotron, SPring-8 using anaesthetised Sprague-Dawley rats 3 weeks after treatment with vehicle (n=8) or streptozotocin (65 mg/kg, i.p. n=11). Induced contrast medium (350 mg/ml) was injected into the coronary circulation. Using cine-radiograms and temporal subtraction we investigated endothelium-dependent and –independent vasodilatory responses in individual coronary vessels. Our results suggest one of the coronary vasodilatory mechanisms is dysfunctional as both segmental vasodilation and focal stenoses were seen after nitric oxide synthase (NOS) and cyclooxygenase (COX) blockade in streptozotocin rats (Fig. 1, black arrows), but not vehicle rats. Thus, in the early diabetic state streptozotocin treated rats show localised coronary impairment. Synchrotron imaging provides a novel method to investigate coronary microvascular function in vivo.
in disease models. These findings further indicate that in the early diabetic state there is already localised coronary endothelial dysfunction.

Figure 1: Synchrotron angiogram depicting non-uniform acetylcholine induced vasodilation of coronary vasculature in diabetic rat after NOS and COX blockade.

**NITROXYL (HNO), A NOVEL REDOX SIBLING OF NITRIC OXIDE (NO), WITH VASOPOTECTIVE ACTIONS**

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The therapeutic utility of the NO/cGMP pathway has long been recognised with nitrovasodilators such as glyceryl trinitrate (GTN) used for the treatment of cardiovascular disorders such as angina, hypertension and heart failure for >100 years. The clinical efficacy of traditional NO donors is limited, however, by susceptibility to metabolic scavenging by superoxide (O$_2^-$) and tolerance development, properties which confer potential for the treatment of heart failure. Importantly, we have also demonstrated that HNO donors are vasoprotective, with potent vasorelaxant and anti-platelet effects and an ability to limit vascular oxidative stress. Thus in rat, mouse and human arteries, HNO donors such as Angeli’s salt and IPA/NO target predominantly the soluble guanylyl cyclase (sGC)/cGMP signaling pathway to mediate vasorelaxation. Moreover, in contrast to GTN, HNO donors do not develop vascular tolerance. Recently, our studies have shown that HNO donors limit oxidative stress, lowering vascular NADPH oxidase-derived O$_2^-$ production in isolated intracranial arteries by ~65%. Such an effect is resistant to inhibitor, ODD and absent in NO-receptor knockout mice. Therefore, we hypothesise that HNO donors modulate NOx-containing NADPH oxidase possibly via a direct, cGMP-independent action. In addition, we have provided the first evidence that the vasoprotective actions of HNO are preserved in disease with the ability of HNO donors to induce vasorelaxation and inhibit platelet aggregation sustained in the setting of hypertension (SHR rats) and hypercholesterolemia (Apoe$^{-/-}$ mice). In conclusion, HNO donors with their favourable vasoprotective properties, lack of tolerance development and preserved bioavailability under conditions of oxidative stress, offer considerable advantages over traditional nitrovasodilators and may provide innovative pharmacotherapy for the treatment of vascular disease.

**HIGH SALT DIET DURING EARLY POSTWEANING LIFE LEADS TO ADULT CARDIOVASCULAR DYSFUNCTION IN ABSENCE OF HYPERTENSION**

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Adult consumption of dietary salt is well in excess of need and has been associated with adverse cardiovascular outcomes. Alarming the intake of dietary salt by young children is also excessive due to the intake of salty snacks. The current study aimed to examine the impact of high salt diet initiated in weanling period on adult cardiovascular health. GDNF Het mice were fed a high salt diet from weaning age to adult age. HNO donors with their favourable tolerability were also subjected to high salt diet to determine whether outcomes would be worsened for those with a reduced nephron endowment. At 20 days of age WT (n = 35) and GDNF Het mice (n = 27) were assigned to receive normal (0.26%) or high salt (5% NaCl) diet. At 10 weeks of age mice underwent 24-h urine collection for measurement of GFR (creatinine clearance) and then had 24-h blood pressure measured by radiotelemetry. Following recordings, hearts and kidneys were excised and abdominal aorta and femoral arteries were collected for assessment of wall stiffness. Bodyweights were unaffected by high salt diet, but kidney weight, kidney to bodyweight ratio and left ventricle to bodyweight ratios were all significantly (P < 0.01) greater in mice on a high salt diet. Mean arterial pressure and GFR were not different between WT and GDNF Het mice on normal salt diets. GDNF Het and WT mice on high salt diets had GFR values ~75% greater than normal salt controls but blood pressures were not different between groups. Wall stiffness in arteries of WT and GDNF Het mice on normal salt were not different but markedly increased (P < 0.0001) on high salt diet. Further, in a subset of WT mice (n = 4) in which the high salt diet was switched to a normal salt diet for 5 weeks, the enhanced stiffness was not reversed. High salt diet initiated at weaning lead to marked kidney and cardiovascular phenotype, renal hypertrophy and vascular stiffening in young adult mice that was not reversed after 5 weeks of normal salt diet. These changes occurred in the absence of hypertension. These findings suggest that greater emphasis should be placed on reducing salt content in food targeted at children and that blood pressure may not be an adequate early marker of cardiovascular risk for those on a high salt diet.

**ARGINASE II DEFICIENT MICE DO NOT DEVELOP NITRATE TOLERANCE: ENHANCED BASAL REACTIVE OXYGEN SPECIES**

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Arginase competes with endothelial nitric oxide synthase (eNOS) for L-arginine, therefore regulating nitric oxide (NO) production in the vasculature. Recently we demonstrated the reduction of nitrate tolerance in the presence of non-isoform specific arginase inhibitors. Here we hypothesise that arginase II inhibition can reduce nitrate tolerance via preservation of intracellular L-arginine and the reduction of reactive oxygen species (ROS). Aorta from wild type (WT; C57BL/6) and arginase II knockout (ArgIIKO) mice were mounted in myographs and assessed using a reduced nephron endowment model. In wild type aorta with 100 μM SNAP, ArgIIKO mice developed increased basal reactive oxygen species (ROS) compared to WT mice (P < 0.05). In contrast, tolerance to GTN in ArgIIKO mice was not different compared to WT mice. In arginase II deficient mice, basal reactive oxygen species were not affected by SNAP or GTN treatment. SNP treatment showed a 32-fold reduction in ROS in ArgIIKO mice compared to WT mice.

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lag are shown in the boxplot figure below. CCP and RAP values from −30 to 20 msec shift appear to be not statistically different to the zero-shifted datasets.

**ASSESSING THE MAGNITUDE OF THE STRUCTURAL TPR-AMPLIFIER IN PAGE HYPERTENSION IN CONSCIOUS RABBITS**

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There has been considerable controversy whether structural changes in the resistance vessels in hypertension enhance vascular resistance (R) responses to constrictor and dilator stimuli. Our group and many investigators have provided evidence from *in vitro*, *in vivo* and model studies. However, others could not confirm experiments pertaining to the total peripheral resistance (TPR) amplifier. To help resolve the controversy we reanalysed an earlier in vivo study 1 in 15 conscious rabbits; both kidneys had been wrapped in cellophane and an aortic Doppler flowmeter for measuring cardiac output (CO) and a left atrial catheter for infusing vasoactive drugs were implanted 5 and 3 weeks before starting experiments. The rabbits were studied on 3 days: 1) with all effectors intact; 2) during ganglionic blockade (GB) with mecamylamine; 3) during neurohumoral block (NHB), which eliminated activity of the ANS and the pressor hormones Ang II and AVP. As agonists we infused AngII, methoxamine, acetyl choline and adenosine, to derive extended scaled dose (ScD) – total peripheral conductance (TPC) and – TPR response curves. Earlier only the TPC curves were examined, and the slope ratio (H/N) between hypertensive and normotensive animals determined by linear regression. As a result we missed two major non-linearities that were more obvious in the TPR curves. On this occasion both curves were examined by polynomial regression, which accounted for 85–95% of their variance. The non-linearities were due to: 1) functional rarefaction associated with reduction of CO at high doses of constrictors; 2) hypotension caused by high doses of dilators, which limited autoregulatory capacity. Between these non-linearities there remained a substantial dose-range for assessing the H:N ratios. TPC and TPR values were calculated from the polynomials over this range for each group at selected ScD intervals. The average H:N ratio is a measure of the amplifier’s magnitude. H:N is best estimated during NHB, when it was 1.84 ± 0.05) without any change occurring during saline infusion (from 18.2 ± 17.1 ± 1.84 mmHg during saline infusion, respectively. Ghrelin, but not saline, induced a rise in plasma glucose concentration (from 4.4 ± 0.1 to 4.8 ± 0.1 mmol/l, *P*<0.05). A stress test consisting of 5-min of forced mental arithmetic was performed following the infusion of saline and ghrelin. During saline, stress induced a significant change in mean blood pressure (+19 mmHg), heart rate (+21 bpm) and MSNA (+35%, *P*<0.05). During ghrelin infusion, the changes in heart rate were less pronounced (+16 bpm, *P*<0.05, compared with saline). Changes in blood pressure and MSNA during the ghrelin infusion were slightly but not significantly reduced compared with saline infusion (+15 mmHg and +11% in MSNA). These results indicate that in healthy human, ghrelin-induced decrease in blood pressure is accompanied by a marked increase, rather than a decrease, in SNS activity. We hypothesize that ghrelin activates the SNS through baroreceptor unloading as a result of a peripheral vasodilator effect rather than by affecting the central nervous system. Furthermore, ghrelin may contribute to the stress induced cardiovascular responses.

**MECHANISM OF ANTI-APOPTOTIC ACTION OF MINERALOCORTICOID RECEPTOR BLOCKADE IN EXPERIMENTAL MYOCARDIAL INFARCTION**

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Heart disease is the leading cause of death in Australia. Although thrombolytic therapy and percutaneous coronary interventions reduce mortality from acute myocardial infarction, additional therapeutic strategies are needed. Clinical trials (RALES & EPHESUS) have shown mineralocorticoid receptor (MR) antagonists added to standard of care substantially increase survival and decrease hospitalization in heart failure. We have previously shown cardiac damage during experimental myocardial infarction is aggravated by the MR agonists aldosterone or cortisol and reversed by 1 μM spironolactone. In addition, spironolactone alone reduced myocardial damage resulting from ischaemia-reperfusion (IR) injury. Since reperfusion injury has been correlated with increased apoptosis in the area at risk, we examined whether receptor activation mediates a cardioprotective effect of MR blockade. Sprague Dawley (SD) rats were anesthetised, the hearts isolated and subjected to regional ischaemia followed by reperfusion. MR antagonists, spironolactone (SP), 1, 3, 10 and 1000 nmol) or eplerenone (EPL 100 and 1000 nmol) were added to perfusates prior to inducing ischaemia and maintained throughout the reperfusion period. At the completion of reperfusion, infarct area and apoptotic index were measured. Apoptotic markers, active caspase-3, annexin, and anti-apoptotic protein, ARC were detected by immunostaining. Spironolactone superfused alone significantly reduced infarct size (35 ± 2%, *P* < 0.01; 1 μmol/l and 36 ± 2%, *P* < 0.05; 10 μmol/l) and the number of TUNEL-positive cardiomyocytes (5.4 ± 0.8%, *P* = 7.7 ± 0.6%, *P* = 0.05) via inverse agonist activity at mineralocorticoid receptors, an effect near-maximal at relatively low dose (10 μM). Although eplerenone (100 nmol and 1 μmol) reduced infarct size (mean values of 37%±2%, *P* = 9–9 and 36%±2%, *P* = 8, respectively) this was not significantly different (p = 0.10 & 0.90). Consistent with the reduction in TUNEL staining, spironolactone reversed both active caspase-3 and caspase processing during IR. Spironolactone also regulated anti-apoptotic protein ARC (apoptosis repressor with a caspase recruitment domain) activity. ARC expression was significantly reduced during IR (1.0 × 10^6±0.9, N=7 versus 2.2 × 10^6±0.3, N=7, *P* = 0.05) and restored


by 10 mM spironolactone (1.9 ± 10^–6 to 0.2, N = 8). Conclusion: Spironolactone acts merely by excluding corticosteroids from mineralocorticoid receptors, but as a protective inverse agonist at low concentration and by activation of anti-apoptotic protein(s).

**050**

**INFLUENCE OF ALTERING DIETARY n-6:n-3 POLYUNSATURATED FATTY ACID RATIO ON MARKERS OF VASCULAR HEALTH IN PATIENTS TREATED WITH TREATMENTS: A RANDOMISED CROSSOVER TRIAL**

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Increasing the dietary intake of n-3 polyunsaturated fatty acids (PUFAs) may decrease the risk of coronary heart disease. However, n-6 PUFAs appear to compete with n-3 PUFAs for common metabolic enzymes, and during incorporation into plasma lipid fractions. This has the potential to modulate the n-6:n-3 ratio in dietary n-6:n-3 ratios. In this, there is a lack of evidence regarding the influence of the n-6:n-3 ratio on vascular function and lipid profile. Subjects treated with statins remain at an elevated risk of cardiovascular disease, and may particularly benefit from such a dietary intervention. Subjects treated with statins (n = 6–8) received two dietary interventions with two different n-6:n-3 ratios (Diet A: 1.7:1 and Diet B: 3:10) in a randomised, crossover fashion. Measures of lipid profile, blood pressure, brachial compliance and sensibility, and pulse wave velocity were determined. Data are presented as mean ± standard error mean using paired t-tests. Both diets caused significant reductions in total cholesterol (Diet A: 4.3±0.7 to 4.3±0.8 mmol/L, n = 6, P = 0.01; Diet B: 5.9±1.5 to 5.0±1.3 mmol/L, n = 8; P = 0.02) and LDL cholesterol (Diet A: 2.9±0.5 to 2.4±0.5 mmol/L, n = 6, P = 0.01; Diet B: 3.6±0.5 to 2.7±0.5 mmol/L, n = 8, P = 0.007). We also observed a significant reduction in systolic and diastolic blood pressure (Systolic: 123.7±6.5 to 113.6±6.8 mmHg, n = 6, P = 0.04; Diastolic: 73.9±4.9 to 69.6±10.1 mmHg, n = 6, P = 0.02) in subjects who received the diet with low n-6:n-3 ratio (Diet A). Other parameters were not affected by either diets (P > 0.10). These results suggest that dietary intervention can markedly reduce LDL cholesterol in patients treated with statins.

**051**

**CENTRAL INFUSION OF RENIN INHIBITOR ALISKIREN PREVENTS SYMPATHETIC HYPERACTIVITY AND HYPERTENSION IN DAHL SALT-SENSITIVE RATS ON HIGH SALT**

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The brain renin-angiotensin system is a major player in salt-induced hypertension. Chronic central blockade of AT1 receptors prevents sympathetic hyperactivity and hypertension in Dahl salt-sensitive (S) rats on high salt. In the present study, we examined whether renin produced in the brain contributes to the salt-sensitive hypertension. A preliminary dose-response study showed that intra-cerebroventricular (icv) infusion of renin A113.6 inhibitor aliskiren B 0.2 and 1 mg/kg/day similarly attenuated salt-induced hypertension without visible adverse effects in Dahl S rats on high salt (8% NaCl, HNa). In the main study, Dahl S rats on HNa or regular salt (0.6% NaCl, RNa) were infused icv with aliskiren (0.2 mg/kg/d) or artificial cerebrospinal fluid (aCSF) as control via osmotic minipumps. One group of Dahl S rats on high salt received a single high-dose of high-energy X-ray (source: LINAC, 6 MV) at either 0 (control) or 5 Gy (irradiated). Between 0 to 24, 24 to 48 hours and 3 weeks following irradiation, rats were anaesthetized (1.3 g/kg, urethane) and beat-to-beat PWV (mmHg) and mean arterial pressure (MAP) measured invasively using 12F catheters and a radiotelemeter (Statham, Inc, Canada) positioned in the descending thoracic aorta via the right femoral and left carotid arteries. Arterial pressure was increased and decreased over the range of 60–150 mmHg with intra-aortic infusion of phenylephrine and sodium nitroprusside respectively. The blood pressure response was measured across the full physiological arterial pressure range, and during the different levels of local neurogenic blockade. Results. The increasing concentrations of phenylephrine caused aortic PWV to increase concordantly. Neurogenic blockade caused a significant change in PWV at arterial pressures above 110 mmHg, with PWV increasing from 5% (10ug/ml phenylephrine) to 10% (100ug/ml phenylephrine) compared to control (P = 0.05). The response was more pronounced at aortic pressures above 130 mmHg, with 100ug/ml phenylephrine resulting in a 12.5% increase in PWV compared to control (P = 0.01). Conclusions. These results show that local neurogenic blockade in large arteries can significantly increase local arterial stiffness. This demonstrates that neurogenic input into large arteries results in functional physiological changes that could impact on blood pressure regulation.

**052**

**SALT-SENSITIVE HYPERTENSION: TIME TO CHANGE THE PARADIGM**

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High dietary salt intake is a major “lifestyle” factor contributing to the progressive increase in BP with ageing in most Western Societies. Despite extensive research, the genetic and mechanical determinants are still poorly defined, in part related to the dogmatic focus on renal mechanisms. Na+-transport regulating mechanisms classically considered to reflect renal control of sodium homeostasis and BP, i.e. aldosterone-mineralocorticoid receptors (MR) –

epithelial sodium channels (ENaC) – Na+/K+-ATPase have now been demonstrated to also be present in the central nervous system. This pathway is being regulated independently of the peripheral/renal pathway and contributes to regulation of cerebral spinal fluid [Na+] by the choroid plexus, of brain tissue [Na+] by the ependyma and to neuronal responses to e.g. Na+ or Ang II. Increases in CSF [Na+] by central infusion of Na+ rich aCSF or by high salt intake in Dahl S. R11005 MR cause sympathetic excitation and hypertension. The aim of this study is to ascertain the exact nature of that mechanism. Looking beyond the kidney is providing new insights into mechanisms contributing to salt-sensitive hypertension, which will help to dissect the genetic factors involved and to discover novel strategies to prevent and treat salt-sensitive hypertension.

**053**

**LARGE ARTERY STIFFNESS INCREASES WITH LOCAL NEUROGENIC BLOCKADE IN RATS**

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Whilst the functional role of neurogenic control of peripheral blood vessels is well established, the presence of neurogenic terminals in the large arteries has not been linked with changes in large artery stiffness. The aim of this study was to elucidate any change in rat aortic stiffness, through measurement of pulse wave velocity (PWV), as a result of local neurogenic blockade. Methods. Male Sprague-Dawley rats (n = 7, aged 12–14 weeks) were anaesthetised and two 1.3F high fidelity pressure sensors introduced into the proximal and distal ends of the abdominal aorta for measurement of PWV. Increasing doses (10ug/ml, 100ug/ml & 1000ug/ml) of an alpha-adrenoregic antagonist (phentolamine) were used to locally chemically denervate the abdominal aorta via bathing through a ventrally exposed cavity. Mean arterial pressure was raised and lowered using intravenously injected phenylephrine and sodium nitroprusside respectively. PWV was measured across the full physiological arterial pressure range, and during the different levels of local neurogenic blockade. Results. The increasing concentrations of phentolamine caused aortic PWV to increase concordantly. Neurogenic blockade caused a significant change in PWV at arterial pressures above 110 mmHg, with PWV increasing from 5% (10ug/ml phenylephrine) to 10% (100ug/ml phenylephrine) compared to control (P = 0.05). The response was more pronounced at aortic pressures above 130 mmHg, with 100ug/ml phenylephrine resulting in a 12.5% increase in PWV compared to control (P = 0.01). Conclusions. These results show that local neurogenic blockade in large arteries can significantly increase local arterial stiffness. This demonstrates that neurogenic input into large arteries results in functional physiological changes that could impact on blood pressure regulation.

**054**

**SINGLE DOSE WHOLE-BODY IRRADIATION CAUSES ACUTE AND REVERSIBLE INCREASES IN LARGE ARTERY STIFFNESS IN RATS**

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Exposure to high energy radiation causes cardiovascular injury. It has been demonstrated that whole-body gamma irradiation impairs the endothelial-dependent vaso-motor function. Such impairment may cause increased arterial stiffness, as can be shown by increased pulse wave velocity (PWV). The current study aims to determine the effects of whole-body high-dose high-energy X-ray on large aortic PW. Male Sprague-Dawley rats (14 weeks old) were exposed to a single high-dose of high-energy X-ray (source: LINAC, 6 MV) at either 0 (control) or 5 Gy (irradiated). Between 0 to 24 hours, 24 to 48 hours and 3 weeks following irradiation, rats were anaesthetized (1.3 g/kg, urethane) and beat-to-beat PWV (mmHg) and mean arterial blood pressure (MAP) measured invasively using two 1.3F high fidelity pressure sensors (SiDiane Inc, Canada) positioned in the descending thoracic aorta via the right femoral and left carotid arteries. Arterial pressure was increased and decreased over the range of 60–150 mmHg with intravenous infusion of phenylephrine and sodium nitroprusside respectively. Across the blood pressure range, mean aortic PW in the irradiated rats measured within 24 hours post-irradiation was 5.0±0.15 m/s, a 14% increase compared to the control group (4.4±0.11 m/s, n = 12; P = 0.05). No significant difference was observed between the PWV in the irradiated group measured in the 24–48 hour period post-irradiation and the control group (4.1±0.10 m/s, n = 4; P = 0.41). Similarly, no difference was found in the arterial stiffness of control and irradiated rats 3 weeks after radiation (control –4.37±0.14 m/s irradiated –4.53±0.13 m/s, n = 12; P = 0.41). Whole-body exposure to a single dose of high-energy X-ray increased aortic stiffness in the period of 0 to 24 hours after the radiation treatment. These changes were reversed in the subsequent 24 hour period, and remained so 3 weeks following irradiation. These findings suggest an acute mechanism behind endothelial impairment associated with whole-body, high-dose irradiation. Further research is required to ascertain the exact nature of that mechanism.
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IDENTIFICATION OF RENIN-ANGIOTENSIN SYSTEM (RAS) IN HUMAN FETAL MEMBRANES, DECIDUA AND PLACENTA AND THE EFFECTS OF GENDER AND LABOUR

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Human intravascular tissues and amniotic fluid contain prorenin. Amniotic (pro)renin can generate Ang I from angiotensinogen (AGT) or stimulate cell signalling pathways directly when bound to the (pro)renin receptor (P)RR. To measure the expression of RAS components, including prorenin, (P)RR, AGT, ACE1, ACE2, AT1R, AT2R, MAS1, amion, chorton, decidua, and placenta were collected before and after labour from 24 women. RNA was extracted and real-time PCR used SYBR Green for detection, with abundance calculated relative to $\beta$-actin mRNA using the 2$^{-\Delta\Delta Ct}$ method. Immunohistochemistry was performed using specific antibodies to localize RAS proteins in membrane and placental sections. Prorenin mRNA abundance was highest in decidua ($P<0.001$), being highest in decidua collected before labour from pregnancies that carried a female fetus ($P=0.06$). After labour, there was a significant decrease in decidua prorenin mRNA in pregnancies carrying a female fetus ($P<0.035$). (P)RR mRNA was highest in placenta ($P=0.04$). AGT mRNA was highest in decidua ($P<0.003$). ACE1 mRNA was lowest in fetal membranes ($P<0.001$) and higher in decidua than in placenta ($P<0.003$). ACE2 mRNA was lowest in fetal membranes ($P<0.001$) and highest in placenta ($P<0.02$). AT1R mRNA was highest in placenta ($P<0.001$). AT2R and MAS1 receptor mRNAs were not detected. It is concluded that in amion, mRNA expression of RAS components is low, but (P)RR is abundant and may bind prorenin from amniotic fluid or decidua. In decidua, RAS components are abundant (except AT1R). In placenta, since ACE1 protein is localized to fetal capillary endothelial cells and AT1R mRNA and protein are present, sycnctrophomblast may contain 2 independent RAS pathways, one in fetal vessels and one in sycnctrophoblasts. The placenta may be affected by maternal Ang II. Therefore fetal membranes may not generate Ang II, and RAS actions may be mediated directly through the (P)RR. Decidua and placenta may be able to generate Ang II and Ang 1–7.

OPTIMISING BLOOD PRESSURE ASSESSMENT IN THE OBSTETRIC DAY ASSESSMENT UNIT

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Obstetric day assessment units (DAU) are used to assess maternal blood pressure (BP) during pregnancy. This method of BP monitoring began to reduce the morbidity associated with hypertension together with diabetes – by 13%. The aim of the study was to compare the symptoms three times higher than in patients without metabolic syndrome. In patients with hypertension, hyperglycemic medication etc. is the increase of patients with type II diabetes, obesity, heart diseases and disruption of lipid exchange. In different countries the rate of people who suffer from metabolic syndrome is 20–45%. In this group of people the level of death is twice higher and level of cardiovascular symptoms three times higher than in patients without metabolic syndrome. In patients with arterial hypertension, function of the renal system decreases by 1% per year, and arterial hypertension together with diabetes – by 13%. The aim of the study was to compare the influence of antihypertension therapy on the speed of glomerular filtration in patients with metabolic syndrome.

Groups received different antihypertensive medication and statins. Groups received different antihypertensive medication and statins. Groups received different antihypertensive medication and statins. Groups received different antihypertensive medication and statins. Comparison of the efficiency of the influence of the anti-hypertension therapy on the speed of glomerular filtration in patients with metabolic syndrome

ENDOTHELIAL PROGENITOR CELLS AND THE CAROTID INTIMA-MEDIA THICKNESS IN SEVERE OBESITY

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Endothelial progenitor cells (EPC) are primitive cells that are important in endothelial repair and regeneration in blood vessels walls following injury. Their impairment is associated with cardiovascular disease (CVD) and risk factors. It is well regarded in non-obese populations that EPC levels serve as a cellular biomarker of CVD. However, EPC levels are not significantly different in severe obesity and may be an important risk factor for EPC. Hence, it is important to understand the mechanism by which EPC levels are regulated in severe obesity.

EPC SIGNALING FOR ANGIOGENESIS IN BRAIN REPAIR FOLLOWING ISAEMIC STROKE AND REPERFUSION

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NADPH oxidase-derived reactive oxygen species (ROS) contribute to the progression of acute brain injury following ischaemic stroke. Despite this, ROS may also regulate endogenous angiogenic mechanisms that occur in the brain after stroke. Angiogenesis in the damaged brain is crucial to support surviving and newly developing neurons. We are searching for molecular targets to enhance these endogenous repair mechanisms that may aid the timely delivery of targeted stem cell therapy. We used rodent models of transient stroke and investigated histological and functional outcomes to determine the timecourse of angiogenesis in the brain following ischaemic stroke. We have also identified endogenous and exogenous signalling factors involved in this response in rats. Functional deficits in behaviour were detected between 1 and 7 days post-stroke ($P<0.01$). Blood vessel numbers decreased within the cortical infarct core 6 h after stroke (30%–10%; $P<0.05$), but by 14 and 28 days numbers increased markedly in the cortical infarct core (64%–4% and 70%–6% respectively) and moderately increased in the cortical border zone (19%–3% and 23–7% respectively, compared to contralateral brain regions ($P<0.05$). Double immunofluorescence labelling revealed that the marked increase in blood vessels in the infarct core at 14 and 28 days was associated with DHE-detectable superoxide generation and only occurred in brain regions that had lost all neurons. Real-time PCR detection of NADPH oxidase (Nox) subunits revealed a marked decrease in Nox2 mRNA expression in the ipsilateral cortex up to 7 days post-stroke (up to 40 fold $P<0.01$) which returned to normal by 14 days. Nox4 mRNA was significantly increased later at 14 days. Angiogenic factor VEGF mRNA increased in the infarct core between 7 and 14 days post-stroke ($P<0.01$) but returned to normal levels by 28 days. Transplantation of stem cells into the brain post-stroke is currently thought to be an exciting treatment option. Optimisation of the damaged brain environment after stroke is crucial for the survival of transplanted cells. NADPH oxidases may be involved in the regulation of angiogenesis in the brain in the weeks following ischaemic stroke and repertusion and therefore present a target for enhancement of brain repair.

COMPARISON OF THE EFFICIENCY OF THE INFLUENCE OF THE ANTI-HYPERTENSION THERAPY ON THE SPEED OF GLOMERULAR FILTRATION IN PATIENTS WITH METABOLIC SYNDROME

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The result of the modern style of life (lack of physical activity, surplus use of high calorie food etc.) is the increase of patients with type II diabetes, obesity, heart diseases and disruption of lipid exchange. In different countries the rate of people who suffer from metabolic syndrome is 20–45%. In this group of people the level of death is twice higher and level of cardiovascular symptoms three times higher than in patients without metabolic syndrome. In patients with arterial hypertension, function of the renal system decreases by 1% per year, and arterial hypertension together with diabetes – by 13%. The aim of the study was to compare the influence of antihypertension therapy on the speed of glomerular filtration in patients with metabolic syndrome.

In the investigation there was no significant difference between the measured variables. During the experiment, the GFR significantly increased in both groups. In the ARB group, GFR increased from 77.92 ± 13.63 ml/min to 91.48 ± 13.63 ml/min ($P<0.03$). In the ACEi group, GFR increased from 75.24 ± 12.97 ml/min to 86.37 ± 11.53 ml/min ($P<0.03$). Comparison of the ARB and ACEi groups after treatment indicated that GFR was higher in the ARB group than in the ACEi group ($P<0.03$). Thus, antihypertensive therapy increases GFR in the setting of metabolic disorder. Interestingly, the results suggest that angiotensin receptor blockade is more effective for increasing GFR than angiotensin converting enzyme inhibition.

ENDOTHELIAL PROGENITOR CELLS AND THE CAROTID INTIMA-MEDIA THICKNESS IN SEVERE OBESITY

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Endothelial progenitor cells (EPC) are primitive cells that are important in endothelial repair and regeneration in blood vessels walls following injury. Their impairment is associated with cardiovascular disease (CVD) and risk factors. It is well regarded in non-obese populations that EPC levels serve as a cellular biomarker of CVD. However, EPC levels are not significantly different in severe obesity and may be an important risk factor for CVD. Hence, it is important to understand the mechanism by which EPC levels are regulated in severe obesity.
and function and carotid intima-media thickness (IMT), an established non-invasive marker of atherosclerosis, in 63 severe obese subjects (46.3 ± 1.1 yrs, BMI 45.2 ± 0.7 kg/m²), and 26 age and gender-matched controls (48.1 ± 2.3 yrs, BMI 25.5 ± 0.5 kg/m²). Circulating EPC level was determined by FACS counting of percentage of AC133+/KDR+ cells in 10⁵ Ficoll-density isolated peripheral mononuclear cells, and EPC colony-forming unit (EPC-CFU) was assessed using a standard EPC-CFU assay. EPC number and EPC-CFU were increased 1.7-fold (0.089 ± 0.011% vs. 0.051 ± 0.011%, P < 0.04) and 3.4-fold (11.1 ± 1.9 colonies/well vs. 3.3 ± 1.1 colonies/well, P = 0.03), respectively, in the severely obese compared to controls. IMT was greater in the severely obese subjects (0.658 ± 0.016 mm vs. 0.570 ± 0.016 mm, P = 0.001), compared to controls. Correlation analysis revealed that EPC number was positively associated with IMT in the controls (P = 0.02) but not in the obese while EPC-CFU did not show any correlation. These results indicate that in severe obesity there is no impairment of EPC function, suggesting that EPC are not an adequate cellular biomarker of CVD in this population. These findings illustrate the complexity of the pathophysiology in the severe obese. Meanwhile the increased EPC number and function may provide a potential cellular mechanism in elucidating a better outcome of established CVD in severe obesity, a mystery of the ‘obesity paradox’.

**MODERATE PRENATAL ETHANOL EXPOSURE IMPAIRS THE PRESSOR RESPONSIVENESS TO STRESS**

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Chronic prenatal exposure to high levels of ethanol can result in impaired fetal development. This may manifest as altered growth, impaired cardiac development and altered sensitivity to stress. Less is known about the impact of more modest ethanol exposure on fetal development. We examined the effects of a moderate prenatal ethanol treatment (PET, 15% calories from ethanol) administered throughout pregnancy in the rat, on fetal and postnatal growth. At 12 months of age, basal blood pressure, heart rate and activity were measured using radiotelemetry and pressor responsiveness to restraint stress was examined. Both males and females were examined. On day 20 of pregnancy, female PET foetuses were growth restricted (n = 8–10 litters/group, P < 0.03). However, there was no effect of PET on body weight of offspring in the postnatal period. At 12 months of age, 24 h mean arterial blood pressure (MAP) in PET offspring was significantly lower than control offspring (n = 6–9 of each sex per group, P < 0.05) although the difference was only 2–4 mmHg. Heart rate was higher in female offspring compared to males (P < 0.001) but there was no effect of PET. Locomotor activity was elevated in female PET offspring compared to control (P < 0.05) but there was no difference between control and PET males. Restraint stress increased MAP by 30–35 mmHg in control animals but only by 20–25 mmHg in PET animals (P < 0.01). In conclusion, moderate ethanol exposure during pregnancy, whilst having minor effects on basal blood pressure, impaired responsiveness to stress. This may involve permanent resetting of the hypothalamic-pituitary-adrenal axis following prenatal ethanol exposure, which is known to be affected by ethanol.

**HEART RATE, BUT NOT BLOOD PRESSURE, RESPONSE TO ENDOTHELIN-1 RECEPTOR BLOCKADE IS GREATER IN GROWTH RESTRICTED FETUSES**

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Small size at birth is associated with an increased risk of perinatal and postnatal cardiovascular morbidity and mortality. Growth restricted fetuses have an increased dependence on the sympathetic nervous system in the maintenance of mean arterial pressure (MAP). Endothelin-1 (ET) mediates cardiac sympathetic innervation by regulating the gene expression of nerve growth factor (NGF). The impact of PET on development of the ET system and the contribution of ET-1 to MAP regulation are not known. The aim of this study was to compare the effect of ET-1 blockade on MAP and HR in the mRNAs expression of NGF, ETA receptor and ETB receptor in control and growth restricted fetuses. Placental restriction (PR) leading to fetal growth restriction was induced by removing the majority of the endometrial caruncles from Merino ewes prior to mating. Vascular catheters were implanted at 120–130d gestation in Control (n = 6) and PET (n = 5) fetuses. Three different doses of endothelin-1 (ET-1) (0.4 μg/kg, 0.8 μg/kg, 1.6 μg/kg) and a single dose of FR139317 (1.5 mg/kg), an ETAR antagonist, were administered intravenously to each fetus and the MAP and HR responses were recorded. In a separate cohort of 9 Control and 9 PET fetuses, coronary arteries were collected at 137–142 d gestation, snap frozen and then the RNA was extracted and real-time PCR as used to measure NGF, ETAR and ETBR gene expression. These data suggest that ET does not play a different role in maintaining blood pressure in the PR fetus and suggest a role for NGF in regulating coronary blood flow in the PR fetus.

**ABIDING – EXTENDING THE UTILITY OF OSCILLOMETRIC BLOOD PRESSURE DEVICES IN GENERAL PRACTICE**

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Peripheral arterial disease is an important contributor to Australia’s burden of disease. A simple method to objectively assess leg arterial perfusion is the Ankle-Brachial Index (ABI). 13,700 oscillometric BP devices have been distributed to Australian GPs by the HBPRCA. ABIDING seeks to expand the utility afforded by these machines by validating them for ABI determination in general practice.

Method: GPs will be recruited through the Victorian REACH database. The Australian REACH Registry [a prospective, observational registry to provide long-term follow-up (38 months) of patients at high risk of athrothrombotic events] consists of 2,782 participants. ABIDING is a cross-sectional validation study. Doppler (by research nurse) and oscillometric (by practice nurse) BP ABI measurements will be performed. The primary outcome is the correlation of ABI defined as the ratio of the highest brachial systolic BP recording to each lower limb systolic BP. Secondary outcomes are: (i) Correlation of baseline ABI and incident adverse CVD events; (ii) Change in ABI, BMI, Edinburgh Claudication score and waist circumference from baseline.

Analyses: Descriptive analyses will be used to investigate the agreement between the two procedures. Sensitivity, specificity, positive predictive value and negative predictive values comparisons between methods for those with abnormal ABI (< 0.9) will be reported. For a sample of 340 we have 80% power to detect a mean difference of 0.05 for our primary endpoint.

Results: In recruitment phase.

Conclusions: Validation of the oscillometric determination of ABI will allow an instrument designed to identify an individual risk factor for CVD (high BP) as a diagnostic tool for generalised arteriopathy (PAD) in primary care.

**AORTIC PRESSURE PULSE AMPLIFICATION IN NORMOTENSIVE, HYPERTENSIVE AND AORTIC CALCIFIED RATS**

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Arterial blood pressure (BP) consists of a steady mean arterial pressure (MAP) and a pulsatile pressure (PP). As the pressure wave travels distally from the heart, MAP and diastolic BP remain essentially constant but a gradual and significant increase of systolic BP and PP occurs. The aim of this study was to characterize the amplification of PP (PPA) between the proximal and distal aortic sites in Wistar Kyoto (WKY), spontaneously hypertensive (SHR) and calcified (VDN) rats. Experiments were performed in anaesthetized (Urethane, 1.3 g/kg, ip) WKY, age matched SHR and VDN rats (n = 6 in each group), Calcification was induced in 8 weeks old WKY with vitamin D3 (300000 IU/kg, im) and 2 doses of nicotine (25 mg/kg, o.p.), one with vitamin D3 injection and the second after 8 hours. Using simultaneous catheterization of proximal and distal aorta with two high fidelity 1.4F catheters, aortic beat-to-beat PPA was recorded over a wide range of BP. MAP was increased and decreased by 30 second infusions of phenylephrine (50 μg/min) and sodium nitrosoguanidine (10 μg/min). PPA is defined as the ratio of the PP recorded at the distal sensor to the proximal. PPA-MAP curves were obtained for pressure range of 50 to 160 mmHg, PPA increased up to the anesthetised operating MAP and then decreased thereafter; PPA was reduced in SHR and VDN compared to WKY at all pressure. Maximal PPA was 1.4 for WKY at a MAP of 90 and 1.15 and 1.16 for VDN and SHR for MAP of 96 and 122 respectively. PPA in all three groups showed a biphasic relationship with MAP. Isobaric PPA was reduced for SHR up to 120 mmHg compared to WKY then converged to unity. PPA of VDN was attenuated to that of SHR but did not converge to WKY suggesting intrinsic changes might have occurred in the vascular properties.

**Figure 1. PPA vs. MAP for SHR, WKY and VDN.**
REDUCED ANGIOTENSIN GENE EXPRESSION FOLLOWING ANGIOTENSIN II STIMULATION OF ASTROCYTES EXPRESSING THE ANGIOTENSIN TYPE 1A RECEPTOR

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Angiotensin II (AngII) acts via AT₁ receptors in several brain regions to regulate blood pressure – one site is the rostral ventrolateral medulla (RVLM) where AngII directly excites sympathetic preotor neurons. Increased angiotensin type 1A (AT₁A) receptor activity in astrocytes of the RVLM leads to a sustained increase in blood pressure (BP) in conscious, freely moving rats. As astrocytes produce the majority of angiotensinogen (Ang) found in the brain, we hypothesized that increased AT₁A receptor activity in astrocytes might increase Ang production, leading to increased local AngII production and sympathetic output. Positive feedback regulation of Ang production by AngII has been demonstrated in vitro. Thus, the aim of this project was to determine whether AngII modulates Ang production by astrocytes. Experiments were performed in primary cultured rat astrocytes. Endogenous expression of AT₁A receptors in primary cultured astrocytes is very low and insufficient to elicit a detectable intracellular signaling response when stimulated with AngII. We increased AT₁A receptor expression by transfection with replication-deficient adenovirus expressing the receptor under control of the ubiquitous CMV promoter. This enabled detection of a robust intracellular signaling response to AngII. Using quantitative PCR we observed that in AT₁A receptor transfected cells, Ang gene expression was significantly reduced by 74.7 ± 3.5% (n = 4; P < 0.005) by AngII (100 nM) stimulation. This effect was blocked by co-administration of the AT₂ receptor antagonist candesartan (1 μM). These novel findings do not support the original hypothesis, but indicate that the AT₁A receptor couples to a negative feedback mechanism to regulate Ang production in astrocytes. Negative feedback regulation of Ang production via the AT₁A receptor has also been reported in cultured cardiac fibroblasts. Further experiments will examine whether this regulation is observed in astrocytes in vivo.

DIFFERENCES IN PERSISTENCE WHEN CALCIUM CHANNEL BLOCKERS ARE COMBINED WITH ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORS

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The NH&MRC Guide to management of hypertension (2008) states that “based on the best available evidence, the most effective combination is an Angiotensin Converting Enzyme (ACE) inhibitor or an Angiotensin II Receptor Antagonist (A2RA) plus a calcium channel blocker (CCB).” PBS claims data provided by Medicare Australia has been used to assess persistence to ACE/CCB (dihydropyridine CCB) combinations. This analysis is based on all scripts supplied to patients in a one in ten sample of the Australian patient population drawn from de-identified Pharmaceutical Benefits data. This analysis is based on all scripts supplied to a one in ten sample of the Australian patient population drawn from de-identified Pharmaceutical Benefits payment records from January 2003 to December 2006. Only Concessional patients were included because many AHT products fall under the General copayment. Initiation was defined as 2 consecutive months of an ACE and a CCB following at least 6 months without an ACE/CCB combination. Treatment cessation was 3 consecutive months of none or just one of the drugs making up the combination. Hazard ratios were derived and adjusted for patient age, sex and initiating specialty. More than 12,000 patients initiated on an ACE/CCB combination, the drugs making up the combination. Hazard ratios were derived and adjusted for patient age, sex and initiating specialty. More than 17,500 patients initiated on an ACE/CCB combination, had their persistence to the combination assessed. Median persistence differed between combinations: lercanidipine/A2RA 23 months [22–25], felodipine/A2RA 20 months [16–22], nifedipine/A2RA 17 months [16–18], and amlodipine/A2RA 14 months [13–15]. Using lercanidipine/A2RA as the reference, patients were significantly (P < 0.01) more likely to cease the other combinations felodipine/A2RA (6.7%), nifedipine/A2RA (18.5%) and amlodipine/A2RA (33.9%). Prescribers need to assess which DHP best supports NIH treatment goals. In terms of optimal treatment persistence, lercanidipine seems to be the best DHP to combine with an A2RA.

DIFFERENT WAYS OF MEASURING COMPLIANCE WITH ANTHYPTENSIVE THERAPY

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PBS claims data provided by Medicare Australia has been used to assess compliance (adherence) with antihypertensive (AHT) therapy. This analysis is based on all scripts supplied to a one in ten sample of the Australian patient population drawn from de-identified Pharmaceutical Benefits payment records from January 2003 to June 2005. Only Concessional patients were included because many AHT products fall under the General copayment. Patients newly initiated to Calcium Channel Blockers (CCB), Angiotensin two Receptor Antagonists (A2RA) or Angiotensin Converting Enzyme (ACE) inhibitors were identified and the following compliance information determined: (1) number of scripts dispensed in the 12 months post initiation, (2) average time between repeats and (3) Medication Possession Ratio (MPR). More than 85,000 concessional patients were initiated on these AHT products. Assessment of compliance found the interval between repeats for AHTs combined was less than 34 days for 75% of patients and MPRs exceeded 90% (considered compliant), while the average number of scripts collected in the first year was only 8.0 per patient (considered non compliant). There were also differences between products. Mean intervals between repeats ranged from 28 (trandolapril) to 44 (captopril) days, while average scripts per year ranged from 3.8 (captopril) to 8.8 (lercanidipine). Using scripts counts over a one year period to assess patient compliance may be misleading since the intervals between repeats suggest that most patients are compliant if they collect their prescriptions. Interventions should be targeted towards ensuring that patients collect their scripts, rather than improving adherence.

CARDIOVASCULAR RHYTHMS AND CARDIAC BAROREFLEX SENSITIVITY IN AT1A RECEPTOR GAIN-OF-FUNCTION MUTANT MICE

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A novel mouse model has been developed that expresses a gain-of-function mutant of the Angiotensin II (AngII) receptor, type 1A (AT₁A). The mutant receptor is constitutively active and has impaired internalisation. These mice have moderately elevated pressure and are more sensitive to lV AngII. The renin angiotensin system contributes to the development of hypertension, not only by a direct vasoconstrictor action of AngII but also via central effects of AngII including an inhibition of baroreflex sensitivity. In this study we evaluated the effects of the AT₁A receptor gain-of-function in cardiovascular rhythms and spontaneous cardiac baroreflex sensitivity (BRS). Transgenic (AT₁A^mut^, n = 5) and wild (AT₁A^WT^, n = 5) mice...
were evaluated using telemetric BP recordings. The gain of the transfer function between systolic BP and pulse intervals used to estimate the spontaneous BRS (ms/mmHg) was calculated in the low frequency (0.15–0.60 Hz) band. Transgenic AT1 MUT higher expressed BP and heart rate (HR) levels compared to controls (systolic BP AT1MUT 134.6±5.9 mmHg; AT1W 110.5±5.8; P<0.05; HR AT1MUT 53±1; AT1W 45.5±5 beats/min; P<0.001). Systolic BP and BRS was dimunition in transgenic AT1MUT 133±3.7 and perennt in AT1W 131±1.7; P<0.05. AT1MUT/AT1W 19.1±0.18 ms/mmHg; P<0.05. Motor activity did not differ between groups. These variables exhibited circadian changes and the differences between the strains were maintained throughout the cycle. The highest values for BP, HR and locomotor activity were observed at night, while the lowest values were observed during the day in both strains. These findings were in line with the observed activ time before adrenalectomy, but also after surgery. One explanation for this finding might be that the changes in the blood pressure and heart rate were influenced by circadian rhythms.

SHOULD TREATMENT FOR GLUCOCORTICOID-SUPPRESSIBLE HYPERALDOSTERONISM (GSH) BE COMMENCED LONG BEFORE HYPERTENSION DEVELOPS, AND, IF SO, WHICH?

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Since GSH can be diagnosed at birth in known families using cord blood DNA, an important question is whether to commence treatment in order to prevent unwanted cardiovascular changes due to aldosterone excess. Since our studies in eight young normotensive affecteds reported five years ago revealed characterized abnormalities compared with age and sex matched normal controls, our objective was to examine the consistency and possible progression of already described disturbances in cardiovascular structure and function in normotensive individuals with GSH. Six of eight subjects with genetically proven GSH who had previously been shown to have structural and functional changes on echocardiography (compared with 24 age- and sex-matched normotensive controls) were restudied after 8.7±14.2 months of follow-up with measurement of office blood pressure (BP) and echocardiographic characteristics, incuding left ventricular (LV) wall thicknesses, parameters of LV diastolic filling and systolic function. Compared with the initial, previously reported data, LV parameters remained similar (130.6±12.7 vs 124.8±15.8 mmHg; P=0.5707) and diastolic BP increased (72.0±9.6 vs 80.3±14.5 mm Hg; P=0.0178). LV posterior wall (0.83±0.09 vs 1.01±0.13 cm; P=0.0185), LV mass (123.9±19.5 vs 190.4±53.7 g; P=0.0153), LV mass index (72.4±8.7 vs 103.6±28.9 g/m2; P=0.0102) and mitral inflow deceleration time (175.5±30.7 vs 203.7±35.6 ms; P=0.0921) increased after follow-up. There were no significant differences in LV diameters and volumes, interventricular septum, ejection fraction, CIVB, E/A wave ratio and E/E' ratio. In GSH, aldosterone excess is associated with increased LV wall thicknesses, LV mass and reduced diastolic function, suggesting that specific treatment (either partial ACTH suppression or aldosterone blockade) should be commenced early and perhaps even long before hypertension develops. Given possible growth-retarding effects of glucocorticoids in children, and lack of receptor specificity of spironolactone, the time has come to discuss optimal treatment with children with GSH.

070 UNILATERAL ADRENALECTOMY IMPROVES URINARY PROTEIN EXCRETION BUT DOES NOT ABOLISH ITS RELATIONSHIP TO SODIUM EXCRETION IN PATIENTS WITH ALDOSTERONE-PRODUCING ADENOMA

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In a previous study we demonstrated that there is a positive association between Uprot and urinary sodium (UNa) in patients with hyperaldosteronism, but not in patients with normal aldosterone levels. The objective of the current study was to determine if Uprot is related to UNa in patients with aldosterone-producing adenoma (APA) and whether the degree of Uprot and strength of this relationship is reduced following correction of hyperaldosteronism by unilateral adrenalectomy. Subjects with APA (n=26) underwent measurement of Uprot and UNa in 24 urine collections before and after adrenalectomy (follow up duration 15.3±12.4 months). Following surgery, mean clinic blood pressure (BP) fell (from 151.7±19.8/85.5±15.3 to 134.3±14.5/83.4±50.5 mm Hg; P=0.001) in the systolic BP; despite a significant fall in number of antihypertensive medications from (1.9±1.2 to 0.7±1.1; P<0.0001). Uprot and urine volume decreased after adrenalectomy (from 201.2±98.3 to 106.8±37.1 mg/dl; P<0.0001 and from 2464.4±854.2 to 2005.3±716.4 ml/day; P<0.0005). There was a positive correlation between Uprot/creatinine ratio and UNa/creatinine ratio both before (r=0.48, P=0.0034) and after surgery (r=0.44, P=0.0242) adrenalectomy. Unilateral adrenalectomy reduces proteinuria in patients with APA. The rapid response in some patients is consistent with a role for hyperfiltration in its etiology. A positive relationship between urinary protein and sodium excretion exists not only in aldosterone excess, but also after surgery. One explanation for this persisting relationship could be long term renal exposure to hyperaldosteronism resulting in glomerular damage and an ongoing tendency to salt-sensitive protein excretion. These findings suggest that both high aldosterone levels and higher dietary salt intake could contribute to increased cardiovascular risk in these patients.

073 OFFSPRING FROM HIGH FAT FEED MOTHERS DISPLAY A SIMILAR CARDIOVASCULAR PHENOTYPE TO DIET INDUCED OBESE ADULT RABBITS

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We have previously demonstrated that offspring from rabbit dams fed a high fat diet (HFD) have elevated mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) compared to control rabbits at 4 months of age. One mechanism by which exposure to a HFD during development may contribute to the development of hypertension is through an elevation in visceral white adipose tissue (WAT) accumulation. The purpose of the present study was to compare RSNA, MAP, HR and WAT accumulation in adult New Zealand White rabbits currently consuming a HFD, to those that were exposed to a HFD during development only (gestation and lactation). After weaning, offspring were fed a restricted control diet. At 4 months of age all rabbits were instrumented with intracerebroventricular (i.c.v.) cannulae and renal nerve electrodes. Body weight was similar between groups, however HFD offspring (n=9) had heavier visceral white adipose tissue compared to control offspring (n=8; P<0.05). Offspring from HFD mothers had an elevated blood pressure, heart rate and renal SNA in comparison to control offspring (P<0.05). Glnalin administration (1civ, 1, 2, 5 and 5 mmol/L) dose dependently decreased blood pressure and heart rate in both groups but elevated renal SNA in HFD offspring only. While the cardiovascular and sympathetic responses to acute airjet stress were similar between groups, i.c.v. administration of glnalin reduced the pressor, tachycardic and renal SNA response to stress in both groups. This inhibition of stress responses was less in offspring from fat fed mothers compared to control (P<0.05). These studies show that the normal sympathoinhibitory actions of glnalin are diminished in offspring from fat fed mothers leading to a greater reactivity to stress during periods when glnalin may be released prior to eating.

071 CENTRAL GHERELIN ADMINISTRATION REDUCES ARTERIAL PRESSURE, HEART RATE AND CARdiovascular Reactivity TO ACUTE AIRJET STRESS IN RABBITS

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Ghrelin is predominantly produced in specific endocrine cells in the stomach and acts in the hypothalamus and brainstem to regulate pre-prandial hunger and meal initiation. More recently, ghrelin has been shown to have peripheral sympathetic actions in cardiovascular function. The aim of the current study was to determine if offspring from obese mothers had altered cardiovascular and sympathetic responses to ghrelin and stress. Female New Zealand white rabbits were fed either a control (3.5% fat) or high fat diet (HFD, 13.5% fat) for 3 weeks prior to mating and throughout gestation and lactation. After weaning, offspring were fed a restricted control diet. At 4 months of age all rabbits were instrumented with intracerebroventricular (i.c.v.) cannulae and renal nerve electrodes. Body weight was similar between groups, however HFD offspring (n=9) had heavier visceral white adipose tissue compared to control offspring (n=8; P<0.05). The cardiovascular and sympathetic responses to acute airjet stress were similar between groups, i.c.v. administration of glnalin reduced the pressor, tachycardic and renal SNA response to stress in both groups. This inhibition of stress responses was less in offspring from fat fed mothers compared to control (P<0.05). Offspring from HFD mothers had an elevated blood pressure, heart rate and renal SNA in comparison to control offspring (P<0.05). Glnalin administration (1civ, 1, 2, 5 and 5 mmol/L) dose dependently decreased blood pressure and heart rate in both groups but elevated renal SNA in HFD offspring only. While the cardiovascular and sympathetic responses to acute airjet stress were similar between groups, i.c.v. administration of glnalin reduced the pressor, tachycardic and renal SNA response to stress in both groups. This inhibition of stress responses was less in offspring from fat fed mothers compared to control (P<0.05). These studies show that the normal sympathoinhibitory actions of glnalin are diminished in offspring from fat fed mothers leading to a greater reactivity to stress during periods when glnalin may be released prior to eating.
term regardless of diet post-weaning. This study highlights how maternal nutrition can detrimentally impact on cardiovascular risk factors in the next generation.

**IS EXERCISE BENEFICIAL FOR BLOOD PRESSURE AND METABOLIC RISK IN OFFSPRING FROM OBESE MOTHERS?**

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Maternal obesity increases offspring cardiovascular risk. Physical exercise ameliorates diet induced fat gain, dyslipidemia and hypertension, and improves insulin resistance. We hypothesized that voluntary exercise would ameliorate the adverse effects of maternal obesity on central appetite regulators, lipid homeostasis and blood pressure in offspring. Sprague-Dawley females were fed either chow (C) or high-fat diet (HFD). Then mated with males. At weaning, female offspring from C dams were fed chow (CC) and offspring from H dams were fed chow (HC) or HFD (HH). Half of each group were provided with a running wheel to enable voluntary exercise (CEx, HCEx, HHEx, n=10–12). Measurements included food intake, blood pressure and glucose tolerance. At week 14, brain, heart, muscle and fat were collected for mRNA measurement of markers for appetite regulation, cardiovascular and glucose and lipid homeostasis. Plasma leptin, insulin, triglycerides (TG), adiponectin and nonesterified fatty acid (NEFA) were determined. HC offspring weighed 12% more than CC offspring (P<0.05). They had increased fat mass, plasma leptin and adiponectin (P<0.05; HC vs CC), which were exaggerated by postnatal HFD (HH vs HC; P<0.01). HFD consumption also increased plasma TG and NEFA with a doubling of food intake and 37% increase in body weight (HH vs HC; P<0.01). Distance travelled on running wheel did not differ across groups. While exercise had no impact in CCEX, exercise reduced the detrimental effects of maternal obesity in both chow and HFD fed offspring. Exercise reduced fat mass, plasma TG and leptin in HHEX vs HH (P<0.01). Blood pressure was elevated in offspring from obese dams consuming HFD (HH vs HC; P<0.001) but this was reversed by exercise (HEx vs HH; P<0.05). Thus maternal obesity and exercise program antagonized each other in part by postnatal HFD. Exercise reduces the deleterious effects of maternal obesity with greater beneficial effects in offspring of obese mothers consuming HFD. Exercise had no obvious effect in offspring of lean mothers consuming a low fat diet.

**MICROGLIA ARE ACTIVATED IN THE HYPOTHALAMUS FOLLOWING MYOCARDIAL INFARCTION**

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Following a myocardial infarction (MI), inflammatory cytokines are elevated in the brain, as well as in plasma, indicating that inflammation is occurring in the brain in addition to the periphery. Microglia are the immune cells in the central nervous system and produce cytokines when they are activated by an insult or injury. In the present study, we investigated whether MI in rats induces activation of microglia in the brain. We used immunohistochemistry to detect CD11b (clone OX-42) and morphological changes to identify activated microglia. Compared to controls, MI rats had undergone a surgical procedure that was a significant stimulus for activated microglia in the hypothalamic paraventricular nucleus (PVN) following myocardial infarction. The increase was observed as early as 2 weeks after the MI and occurred predominantly within the paraventricular PVN. Activated microglia were not observed in the ventral hypothalamus, adjacent to the PVN, nor in the cortex, indicating the response was not the result of a generalised inflammatory reaction in the brain. Echocardiography and haemodynamic parameters 2 weeks after myocardial infarction indicated reduced left ventricular function but congestive heart failure had not yet developed. In conclusion, microglia are activated in the PVN but not in the adjacent hypothalamus following myocardial infarction. The activated microglia may contribute to the increased local production of pro-inflammatory cytokines observed in the PVN after myocardial infarction and resulting reduced left ventricular function.

**LOST THERAPEUTIC BENEFIT (LTB) IN HIGH RISK PATIENTS MANAGED FOR HYPERTENSION IN AUSTRALIAN GENERAL PRACTICE**

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Background: Hypertension is a common risk factor for cardiovascular disease and despite the widespread use of anti-hypertensive medication, many people remain uncontrolled (Blood Pressure [BP] >130/80 mmHg). Lost Therapeutic Benefit (LTB) (receiving medication without attaining target BP levels) may lead to increased morbidity and mortality due to cardiovascular disease.

Aim: To estimate the extent of LTB in patients at high risk of atherothrombotic events and to model the impact of attaining target BP levels in LTB patients on cardiovascular event rates over a two year period.

Methods: The Australian REACH registry consists of 2872 high risk patients of which 2856 (99.4%) were followed for cardiovascular events over a two year period. The mean age was 72.8±9.9 yrs, 65.1% were male and 78.7% had a history of hypertension. LTB was calculated as the proportion of patients receiving antihypertensive therapy who were not attaining guideline BP control targets. A hypothetical intervention to lower blood pressure to the normal range was applied to those individuals identified with LTB, to estimate the number of cardiovascular disease events (cardiovascular disease death, non-fatal stroke and non-fatal MI) which could be prevented. Logistic regression was used to predict the finding of LTB amongst patients with controlled and uncontrolled BPs. Univariate and multivariate analyses were performed with age, gender, diabetes, hypertension, carotid plaque, cholesterol, BMI and congestive heart failure. The cardiovascular event rate in those people with LTB was 6.9% while those on medication and controlled BP had a event rate of 4.4%. Assuming a hypothetical blood pressure intervention is applied to the LTB group resulting in controlled blood pressure (<130/80 mmHg), 8 cardiovascular events (CV death, non-fatal stroke, and non-fatal MI) per 1000 people and 21 cardiovascular disease events including coronary heart disease intervention such as CABG, coronary angioplasty, carotid surgery, etc. per 1000 people could be prevented.

Discussion: Improving BP control in patients receiving antihypertensive medication may prevent 8 cardiovascular events per 1000 people and 21 CVD events per 1000 people within this study group. At a population level, this would represent a major cardiovascular event reduction strategy.

**THE POWER OF THE MORNING BLOOD PRESSURE SURGE AND ITS RELATION TO LONG-TERM SURVIVAL IN THE 2ND AUSTRALIAN NATIONAL BLOOD PRESSURE STUDY (ANBP2)**

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Objectives: Cardiovascular risk is greatest in the morning period. We defined a new measure of the morning blood pressure surge which is derived from ambulatory blood pressure monitoring (ABPM) by the product of the rate of morning rise (RoR) and the amplitude (day-night difference) giving an effective “Power” of the blood pressure rise (BPPower). We have examined the association of morning BP-power and heart rate and long term clinical outcomes in elderly hypertensive patients undergoing ambulatory blood pressure monitoring in ANBP2.

Methods: BP-power was calculated using a double logistic fitting procedure from 712 ambulatory recordings from male and female subjects. Five-year follow-up (short-term) was conducted by study nurses and 10 year follow-up was conducted via record linkage with the National Death Index with mail and telephone follow-up of survivors. Cox-proportional hazards models were used to determine the association of BP-power on short- and long-term survival.

Results: Forty-two and 130 (18.3%) deaths accumulated over a medium- and long-term follow-up of 4.1 and 9.2 years respectively. After adjusting for age and sex, systolic BP power in the lowest quartile was associated with a 42% greater long-term risk of death in comparison to the highest quartile (OR 1.35; 95% CI: 0.82 – 2.23; P=0.20). Diastolic BP Power had no impact on survival (OR 1.03; 95%CI 0.63 – 1.68; P=0.90) However for heart rate power the reverse was observed. Subjects with the lowest heart rate power had a 23% lower risk of death (OR 0.77; 95% CI 0.48 – 1.46; P=0.29).

Discussion: These trends support the hypothesis that blood pressure power and heart rate may be markers for survival outcomes in elderly hypertensive patients.

**IS CATHETER-BASED RENAL DENERVATION ASSOCIATED WITH A SUSTAINED BLOOD PRESSURE REDUCTION IN PATIENTS WITH RESISTANT HYPERTENSION? COMPLETE 12 MONTHS SAFETY AND EFFICACY RESULTS**

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Hypertension is a global public health problem of major magnitude; an estimated 30–40% of the adult population in the developed world suffers from this condition. Despite the availability of pharmacologic therapies, only half of treated patients are controlled to established targets. Renal sympathetic hyperactivity is central in the progression of hypertension. Catheter-based renal denervation has recently shown promise in treating this target, and thus lowering blood pressure (BP). Renal denervation using a radiofrequency catheter (Symplicity Catheter System, Aridian Inc., Palo Alto, CA, USA) was tested in patients with resistant hypertension (systolic BP ≥160mmHg on ≥3 anti-hypertensive medications, including a diuretic). Office BP and safety data was acquired at baseline and at 1, 3, 6, 9 and 12 months post-procedure. Fifty patients were enrolled at 5 centers in Australia and the EU; 5 patients were excluded for anatomic ineligibility pre-procedure. Among treated patients, mean age was 58±9 yrs, 44% were female, 31% diabetic and 22% had coronary artery disease. Baseline office BP was 177/101±23 mmHg, systolic and diastolic blood pressure being significantly lower in LTB patients. Depression of considerable relevance given the theoretical potential for the BP-lowering benefits of the procedure to wear off over time due to the potential of re-growth of ablated nerve fibers.
**RENALINE PLASMA LEVELS ARE ASSOCIATED WITH SYSTOLIC BLOOD PRESSURE IN PATIENTS WITH RESISTANT HYPERTENSION**

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Evidence from experimental studies suggest that renaline, a soluble FAD-dependent protein, is involved in blood pressure regulation, possibly via degradation of catecholamines including noradrenaline. To investigate whether renaline is associated with blood pressure levels and/or index of large vessel stiffness in humans we compared a cohort of 22 patients with resistant hypertension (at least 3 antihypertensive drugs including a diuretic) and 4 healthy, normotensive control subjects. Radioisotope dilution methodology and arterial blood sampling was applied to measure total body noradrenaline (NA) spillover. Arterial plasma levels of renaline were measured by Western blot analysis using a monoclonal anti-renaline antibody and quantified using a gel documentation system (Bio-Rad Quantity One Software). Split half analysis of the hypertensive cohort according to systolic blood pressure levels (mean: 168±22 vs 156±5mmHg; P<0.001) revealed that mean arterial renaline levels were substantially lower in the patients with higher systolic blood pressure (85.31 vs 125.82 arbitrary units; P<0.05), whereas whole body NA spillover tended to be higher in the group with higher systolic blood pressure without reaching statistical significance (845±445 vs 407±195mm/min; P=0.12). Arterial renaline levels were higher (238±174 arbitrary units) and whole body NA spillover was lower (168±78ng/min) in the normotensive control subjects (mean systolic blood pressure: 123±7mmHg/81±5mmHg, P<0.05). Correlation analysis revealed an inverse relationship between arterial renaline plasma levels and systolic blood pressure for the entire cohort (r=−0.52; P=0.05). These data suggest that arterial plasma levels of renaline are inversely associated with systolic blood pressure in a cohort of patients with resistant hypertension. Whether this relationship, in part be explained by alleviated degradation of noradrenaline or whether alternative pathways are involved requires further investigation.

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**MEASUREMENT OF BLOOD PRESSURE DURING A SINGLE BOUT OF LOW INTENSITY EXERCISE IDENTIFIES PATIENTS WITH MASKED HYPERTENSION**

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Masked hypertension (MH) is an independent predictor of cardiovascular mortality, but cannot be diagnosed from blood pressure (BP) taken in the clinic. We sought to determine if MH could be identified from BP or pressure waveform analysis (PWA) either at rest or during a single bout of low intensity exercise. Brachial and estimated central BP by PWA (SphygmoCor) were recorded at rest and during ~10 minutes of light intensity cycling exercise (60–70% of age-predicted maximal heart rate) in 77 untreated subjects with a hypertensive response to exercise (HRE) (aged 54±8 years) and 61 patients with hypertension (HT) receiving antihypertensive therapy (aged 61±7 years). All subjects underwent assessment of aortic and brachial arterial stiffness via pulse wave velocity (PWV) in addition to 24 hour ambulatory BP monitoring (24ABPM). MH was defined as clinic systolic BP (SBP) <140mmHg and 24ABPM SBP ≥130mmHg. There were 44 (58%) HRE and 32 (42%) HT patients with MH. For the HRE group at rest, there were no significant differences between MH and normotensive subjects in any haemodynamic variable except brachial systolic BP which was higher in MH subjects (127±9 vs. 120±8 mmHg; P<0.05). After correction for resting SBP, MH subjects had significantly higher brachial (167±22 vs 168±15 mmHg; P=0.05) and central SBP (154±17 vs. 141±12 mmHg; P=0.05) during exercise, with greater changes in both from baseline (P=0.05). No differences were observed in the HT group. A binary logistic regression model vs. 141

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**SPIRONOLACTONE IMPROVES LIGHT EXERCISE CENTRAL SYSTOLIC LOADING IN SUBJECTS WITH EXAGGERATED EXERCISE BRACHIAL BLOOD PRESSURE: A RANDOMISED CONTROLLED STUDY**

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Exaggerated exercise blood pressure (EEBP) predicts mortality. The mechanisms of this association are unknown but have been linked to increased central arterial stiffness and raised central blood pressure (CBP) during low-stress physical activity. Spironolactone has antihypertensive effects that may reduce large artery stiffness and lower exercise central BP. This study aimed to test these hypotheses. Untreated patients without hypertension or coronary artery disease, but with EEBP (N=112; aged 55±6 years, 58% male), were randomised to 3 months spironolactone (52 mg daily; n=57) or placebo (n=55). An EEBP was defined as brachial BP ≥190/105 mmHg (women) or ≥210/105 mmHg (men) during maximal exercise. Arterial stiffness was recorded by aortic pulse wave velocity (PWV). Brachial BP was estimated by sphygmonanometer and central BP by radial tonometry at rest, during low-stress physical activity (cycle ergometry at 60–70% maximal heart rate) and after maximal exercise. Patients also underwent 24 hour ambulatory BP monitoring (24ABPM), VO2max testing and 2D echocardiography for left ventricular (LV) structure and function. At baseline, aortic PWV was associated with peak exercise systolic BP (SBP) (r=−0.24; P=0.01), low-stress central pulse pressure (r=−0.23; P=0.03) and VO2max (r=−0.29; P=0.003). Compared with placebo, spironolactone significantly reduced 24ABPM SBP (r=−3.8±7.3 versus 1.0±8.7 mmHg; P<0.004), maximal exercise brachial SBP (r=−3.3±16.3 versus −0.5±11.0 mmHg; P<0.002) and maximal central SBP (r=−7.6±11.6 versus −1.3±10.5 mmHg; P<0.007) but did not change aortic PWV, VO2max or LV parameters (P>0.05 for all). Moreover, central SBP (r=−4.1±12.1 versus 0.7±13.1 mmHg; P=0.03) and the systolic pressure-time integral (r=−230±477 versus −111±419 mmHg) were significantly reduced during low-stress exercise (P<0.05 for both), whereas low-stress central SBP was unchanged by spironolactone (r=−3.8±16.3 versus 1.0±13.0 mmHg; P=0.14). We concluded that maximal exercise BP, as well as submaximal central systolic loading during light activity, are improved by spironolactone in patients with EEBP, but these changes cannot be attributed to reduced central artery stiffness.
function in obese metabolic syndrome subjects

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The metabolic syndrome (MetS) is independently associated with an increased risk for incident chronic kidney disease. The objectives of this study were to examine (1) the effects of lifestyle intervention on renal function in this clinical setting and (2) correlates of improved renal function. Thirty-eight MetS subjects (23 M, 15 F; mean age 55 ± 1.1 year) were randomly randomized to a lifestyle intervention group (n = 20, mean change in waist circumference = 24 ± 10 cm, 2 ml/min/1.73m2 in WL) or a control group (n = 18, P = 0.05). All parameters remained unchanged in the control group. Change in GFR correlated inversely with P(0.04) between groups). Fitness (maximal oxygen consumption) increased by 15±5% in the WL group (P = 0.05). Estimated GFR increased in both lifestyle groups (from 88±2 to 92±2 ml/min/1.73m2 in the WL group and 89±2 to 96±2 ml/min/1.73m2 in the WL + EX group, P = 0.001), however the WL + EX group lost more body weight (–10.4±1.1 versus –7.9±0.8 kg) and trunk fat mass (–5.3±0.7 versus –3.4±0.6 kg, P = 0.05 between groups). Fitness (maximal oxygen consumption) increased by 15±5% in the WL + EX group (P = 0.05). Estimated GFR increased in both lifestyle groups (from 88±2 to 92±2 ml/min/1.73m2 in the WL group and 89±2 to 96±2 ml/min/1.73m2 in the WL + EX group, P = 0.001), however the increment was greater in the latter (P = 0.04 between groups). In the subset of subjects with albuminuria, urinary albumin excretion decreased from 46±31 to 16±28 mmol/day in the WL group (n = 8, P = 0.01) and from 31±4 to 15±5 mmol/day in the WL+EX group (n = 4, P < 0.05). All parameters remained unchanged in the control group. Change in GFR correlated inversely with change in waist circumference (r = −0.74, P = 0.001), systolic blood pressure (r = −0.32, P = 0.08), diastolic blood pressure (r = −0.40, P = 0.02) and positively with change in whole-body insulin sensitivity index (r = 0.54, P = 0.001) and fitness (r = 0.38, P = 0.04). In conclusion, moderate weight loss is associated with an improvement in estimated GFR and a reduction in albuminuria in obese MetS subjects. Exercise training may augment these effects, possibly by promoting greater central fat loss.

Effect of Hydrogen Sulphide in the Brain on Cardiovascular Regulation

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Hydrogen sulphide has long been known for its smell and toxicity. In the last decade, however, hydrogen sulphide has been found to have several physiological effects including vasodilatory and neuromodulatory roles. In the cardiovascular system, hydrogen sulphide has been shown to induce smooth muscle relaxation in the periphery, and to inhibit neuronal excitability in the brain. Recently it has been suggested that hydrogen sulphide acts within the brain to reduce blood pressure. In the present study we have investigated the effects of microinjecting hydrogen sulphide donors and the effects of inhibiting endogenous hydrogen sulphide production on blood pressure (BP), heart rate (HR) and lumbar sympathetic nerve activity (LSNA) in anaesthetised Wistar-Kyoto rats. The animals were kept on a day-night cycle using artificial timed light from 6 am - 6 pm daily. The MSBP was defined as the BP 2 hours after rising (8 am) minus the average BP overnight (6 pm - 6 am). Data from 2 mornings and nights was averaged, and t-tests were performed for statistical analysis using PASW Statistics 17.0 software (SPSS Inc., USA). There were no significant differences between the groups in age or weight of the animals. In the non-pregnant animals, mean overnight BP was 127±2 mmHg systolic and 83±2 mmHg diastolic and morning BP was 153±4 mmHg systolic and 90±1 mmHg diastolic. In the pregnant animals the MSBP was 112±4 mmHg systolic (P = 0.004) and 76±3 mmHg diastolic (P = 0.101) and in the morning (122±3 mmHg systolic (P = 0.028) and 79±3 mmHg diastolic (P = 0.044)). The mean DBP was 12±3 mmHg systolic and 7±2 mmHg diastolic for the non-pregnant baboons, and 10±2 mmHg systolic and 3±2 mmHg diastolic for the pregnant baboons (not significantly different, P = 0.699 and 0.085, respectively). All results suggest that baboon blood pressure is similar to humans in terms of the MSBP, with a rise in systolic BP of 10 – 15 mmHg in the morning. Pregnancy does not appear to affect the presence or magnitude of the MSBP. Also similarly to humans, the blood pressure of pregnant females is significantly lower than that of non-pregnant females.

TUMOR NECROSIS FACTOR ALPHA INDUCES PLACENTAL ANTIINFLAMMATORY FACTORS IN PREGNANT BABOONS (PAPIO HAMADRYAS)

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We have previously reported that TNF-α infusion increases blood pressure, induces proteinuria and increases soluble FMS-like tyrosine kinase 1 (sFlt-1) plasma concentrations in pregnant baboons. The aim of the current study was to determine if the source of increased circulating sFlt-1 in this model of preeclampsia (PE) is the placenta, and to determine if there was increased placental expression of endoglin (Eng), another angiogenic factor implicated in PE. Telemetered mid-trimester pregnant baboons were given an infusion via osmotic minipump (1.7 ng/kg/day) (n = 3) for 2 weeks. The progesterone infusion increased sFlt-1 plasma concentrations in the evening (P = 0.085) and non-pregnant (n = 3) female baboons had a telemetered mid-trimester pregnant baboons. Infusion of TNF-α did not affect the cardiovascular variables measured compared to vehicle. In separate groups of rats in which the NaHS (0.2–2000 pmol, n = 5), or the inhibitors, hydroxyamine (0.2–2 mmol, n = 5) and amino-oxyacetate (0.1–1 nmol, n = 5), inhibited of cystathionine beta synthase, an enzyme responsible for the production of hydrogen sulphide, were administered into the PVN, neither drug significantly affected the cardiovascular variables measured compared to vehicle. In separate groups of rats in which the NaHS (0.2–2000 pmol, n = 5), or the inhibitors, hydroxyamine (0.2–2 mmol, n = 5) and amino-oxyacetate (0.1–1 nmol, n = 5), were microinjected bilaterally into the pressor region of the RVLM, there was no significant effect on BP, HR and LSNA compared to vehicle controls. At the end of each experiment the injection sites in the brain were confirmed by histology. The results suggest that hydrogen sulphide in the hypothalamic PVN or the RVLM does not play a major role in the regulation of the cardiovascular system.
THE RECRUITMENT OF BLOOD-BORNE QDOT-LABLED CELLS INTO ATHEROSCLEROTIC PLAQUE

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We have come full circle is determining whether bone marrow or blood born precursor cells can enter the diseased vessel wall to form new endothelial or smooth muscle cells. Thus, we sought to determine whether blood-borne cells could be recruited during an atherogenic diet. Rabbits were injected for four days with 15μg/day GFSF and on the fifth day, CD45- blood borne cells (characteristic of stromal lineages) were isolated, labeled with QDots, and stored at −70°C. Then, the same rabbits were fed and atherogenic diet for 4 weeks and 10th QDot-labeled autologous cells were injected weekly during this time. Viability of cells in vivo was confirmed the day after using flow cytometry. Labeling peptide, Ang (1–7) with the ascending aorta and coronary artery (LMA) were then studied by confocal microscopy. QDot-positive ‘neo’ endothelial cells were visible in both the ascending aorta and the LMA, which appeared to be concentrated on the shoulder of the plaque in the LMA. Moreover, QDot-positive neo-intimal cells and medial cells were also found in the LMA and not the ascending aorta. There were also QDot-negative cells throughout both vessels. Conclusions: Blood borne CD45-negative cells form part of the neo-endothelial layer during an atherogenic diet, but only form neo-intimal and medial cells in the LMA at this time point. These studies strongly support the hypothesis that blood born cells can be recruited into atherosclerotic plaque, and that they appear to be concentrated in the ‘shoulder’ region of plaque.

HEART RATE-DEPENDENCE OF AORTIC PULSE VELOCITY VELOCITY IN RATS

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Aortic pulse wave velocity (aPWV), a surrogate measure of arterial stiffness, is a strong independent predictor of cardiovascular disease and all-cause mortality. Whilst studies have shown associations between changes in arterial stiffness with acute changes in heart rate (HR), the effect of HR on aPWV at different mean arterial pressures (MAPs) have not yet been fully investigated. In this study, aPWV was measured in age-matched (12 weeks), urethane-anesthetized normotensive rats (n=6) at different HRs across a wide MAP range (40–160 mmHg). HR changes were achieved by atrial pacing at randomly sequenced rates (350, 400 and 450 bpm) after the resting HR was lowered with multiple doses of a bradycardic agent (zatebradine, 2 mg/kg i.v.). aPWV was measured by inflating phenylephrine and sodium nitroprusside respectively (30 μg/kg/min i.v.) Effects of HR on aPWV were analysed at each low (40–80 bpm), medium (80–120 mmHg) and high (120–160 mmHg) MAP range. Data are presented as mean ± SE and Student’s 2-tailed H-test for paired observations was applied to compare means of aPWV at different HRs within the same MAP range. HR is shown to have no significant effects on aPWV at the low and medium MAP ranges, but a significant effect was seen at high MAPs. Low MAP Medium MAP High MAP

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*p<0.05 was considered statistically significant.

ATHERO-PROTECTIVE EFFECTS OF THE ACE INHIBITOR, PERINDOPRIL, ARE PARTIALLY MEDIATED VIA ACTIVATION OF BOTH MAS AND AT1 RECEPTORS IN APOPIOPROTEIN E-DEFICIENT MICE

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It is now well recognized that ACE inhibitors mediate athero-protective effects, both experimentally and clinically, however it is hypothesized that these protective effects may not just be due to inhibiting formation of Angiotensin (Ang) II as studies have shown a concomitant increase in levels of the anti-angiotensin peptides pepitase (Ang). We have previously reported that Ang (1–7) can stimulate non-AT, receptors, i.e., AT1 and Ang (1–7)/Mas receptors to produce athero-protective effects, thus the aim of the current study was to determine whether athero-protective effects produced by the ACE inhibitor, perindopril, are also mediated via these two receptors. Oncogenic treatment was examined using ApoE−/− mice fed an atherogenic high fat (21%) diet for 16 weeks. During the final 4 weeks ApoE−/− mice received one of the following treatments: perindopril (4mg/kg/day); vehicle (saline); perindopril in combination with either the AT1-R antagonist, PD123329 (1 or 10mg/kg/day), or the MasR antagonist, A779 (48mg/kg/hr); PD123319 or A779 alone. Chronic perindopril treatment significantly reduced blood pressure, inhibited atherosclerotic lesion development and stabilized atherosclerotic plaque as measured by increased collagen formation and decreased lipid deposition within the brachiocephalic artery. These antiatherosclerotic and atheroprotective effects were reversed with co-treatment with either the AT1-R or the MasR receptor antagonist. Restoration of NO bioavailability appears to play a significant role in the athero-protective effect mediated by perindopril treatment as eNOS immunoreactivity and protein levels were significantly increased whilst superoxide levels were significantly decreased, with both effects of perindopril reversed with either AT1-R or MasR receptor blockade. In conclusion, we have shown for the first time that the athero-protective effects of the ACE inhibitor, perindopril are at least partially mediated by activation of both AT1-R and Mas receptors, possibly involving stimulation by the angiotensin peptide fragment, Ang (1–7). This study highlights the importance of the Ang (1–7)/Mas/AT1-R axis which acts as a counter-regulatory pathway against the pro-atherogenic ACE/Ang II/AT1-R axis.
AORTIC STIFFNESS, BUT NOT CENTRAL OR BRACHIAL BLOOD Pressures, PREDICT PHYSICAL QUALITY OF LIFE

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Unless severe and uncontrolled, hypertension is common asymptomatic. However, some studies suggest that lower brachial blood pressure (BP) in patients with uncomplicated hypertension may improve quality of life (QOL). The relation between central BP and QOL has never been assessed, but may be relevant because large differences in central systolic BP (SBP) can occur between individuals with similar brachial SBP. This discrepancy between central and brachial SBP is mostly attributed to individual variation in large artery stiffness. We sought to determine the relation between QOL BP (brachial and central) and large artery stiffness in 104 patients receiving therapy for uncomplicated essential hypertension (aged 63 ± 8 years, 53% male) who were free from a history of cardiovascular or renal disease. The SF-36 health survey was used to quantify QOL. Brachial BP was assessed in the clinic (whilst seated and standing), at home (7 day average) and by 24 hour ambulatory monitoring (24ABPM). Clinical central BP and aortic pulse wave velocity (PWV) (for arterial stiffness) were estimated by applanation tonometry. Neither brachial nor central BP’s were associated with QOL measures (P>0.05 for all). However, physical functioning scores significantly declined across tertiles of aortic PWV (tertile 1; 89±14, tertile 2; 76±22, tertile 3; 76±25) even after correcting for age, gender, clinic brachial SBP and 24ABPM SBP (ANCOVA P=0.028). On multiple regression analysis, aortic PWV (but no BP measure) independently correlated with physical functioning (β = −0.26; P=0.012), but only accounted for 6% of the variance in this QOL measure. We conclude that physical well being is negatively associated with large central artery stiffness, which provides further evidence that interventions to reduce arterial stiffness may improve patient outcomes. Furthermore, central BP appears to offer no additional information beyond brachial BP regarding QOL. Whether this finding will be supported by the use of a hypertension-specific QOL survey needs to be assessed.

KYNURENINE IS A NOVEL ENDOTHELIUM-DERIVED VASCULAR RELAXING FACTOR IN Atherosclerosis

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Indoleamine 2,3-dioxygenase (IDO) is a heme-containing enzyme that metabolizes the essential amino acid tryptophan to kynurenine. In acute inflammatory diseases, including malaria and sepsis, IDO is induced and contributes to hypotension. We investigated whether IDO plays a role in the regulation of vascular tone in atherosclerosis, a disease associated with chronic inflammation. The expression of IDO protein was determined in atherosclerotic lesions of ApoE−/− single and ApoE−/−Ido−/− double gene knockout mice, and in human carotid plaques by immuno-histochemistry. Synthesis of the tryptophan metabolite kynurenine was recovered in conscious mice using a computerized, non-invasive tail cuff system. Vessel function was assessed in vitro using a standard myograph system. IDO was expressed in atherosclerotic lesions of ApoE−/−mice and humans, whereas the protein was absent in non-diseased arteries or the arteries from ApoE−/−Ido−/− mice. Pharmacological inhibition of IDO by its competitive inhibitor 1-methyltryptophan increased blood pressure in ApoE−/− but not in ApoE−/−Ido−/− mice. In the myograph system, addition of tryptophan caused a relaxation in the pre-constricted aortic rings from ApoE−/−, but not ApoE−/−Ido−/− mice. Also, kynurenine relaxed aortic rings in an endothelium-independent manner, whereas other known kynurenine pathway metabolites had no material effect on vessel relaxation. Arterial relaxation by Kyn was mediated by activation of the adenylyl and soluble guanylate cyclase pathways. This study suggests that tryptophan metabolism to kynurenine may contribute to the regulation of vascular tone in atherosclerosis, opening the possibility for novel treatments of ischemic complications arising from atherosclerosis.

COST BENEFIT OF SALT REDUCTION TO COMPLEMENT EXISTING CLINICAL HYPERTENSION PROGRAMS

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Excess dietary salt is a key contributor to high blood pressure and vascular disease. National and international organisations recommend that average population salt intakes are reduced to below 6g per day. The objective of this study was to compare the likely costs and benefits of adding a national salt reduction program to existing clinically based disease prevention strategies. We undertook a search for studies reporting the actual or projected costs and/or effects of hypertension management and salt reduction in Australia, summarised the data and compared the costs and effects of the two strategies. We found that hypertension management in Australia costs ~$1 billion a year (~$500 million on drug therapies and $500 million on other costs) and likely prevents approximately 10% of the disease burden attributable to high blood pressure. A national salt reduction strategy to reduce average daily intakes from 9g to 6g in Australia was projected to cost ~$15 million dollars a year, (~2%-3% of the hypertension program). This would reduce population blood pressure levels by 3–4mmHg, resulting in a 15–20% reduction in premature vascular disease in the immediate term and a 20–30% reduction in the longer term, due to the cumulative effects of the attenuation of the population rise in blood pressure with age. The costs of the salt reduction program are anticipated to decrease over time. Expansion of hypertension recognition and treatment would also prevent more events but would be less cost effective. In conclusion, there appears to be a strong case for the addition of a nationally coordinated salt reduction program to the existing clinical hypertension control program with substantial health benefits accrued at minimal additional cost.

EDHF RESPONSES IN MUSCLE RESISTANCE ARTERIES FROM TYPE-1 DIABETIC RATS

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The endothelium-derived relaxing factors, endothelin-derived hyperpolarizing factor (EDHF) and nitric oxide (NO), can invoke endothelium-dependent hyperpolarization and relaxation of the vascular smooth muscle. This study investigates the effects of 10-week streptozotocin (STZ)-induced type-1 diabetes on endothelial function in the cromaster artery, a skeletal muscle resistance artery, compared to corresponding control rats. In MOPS (3-[N-morpholino]propanesulfonic acid)-modified Krebs buffer containing 10 μM indomethacin, endothelium function was assessed using pressure myography by measurement of the internal diameter of isolated segments of cremaster arteries, maintained at a physiological pressure of 30 mmHg, with changes in diameter recorded using a video image processor and analysed with a computerized analysis program. To model the effects of age-related arterial stiffening in the cremaster, arteries were exposed to increasing concentrations of endothelin-1 (ET-1) during each recording, the internal diameter of the response being measured at 30 and 120 s after exposure to ET-1, with responses to the NO donor, sodium nitroprusside. The results show that the ET-1-mediated relaxation was significantly reduced in arteries from diabetic rats as compared to control rats. This is consistent with the observations that diabetes induces arterial stiffening and suggests that the function of the ET-1 system is compromised in diabetes.
70 mmHg. Blood glucose was significantly greater (P<0.05, Student’s t-test) in diabetic rats (33.0±0.2 mM, n=22) than in control rats (11.9±0.5 mM, n=20). Acetylcholine (ACH)-induced vasodilation, but not levocarnitamol (LK)-induced vasodilation, was significantly attenuated (P<0.05 ANOVA) in diabetic rats (E_max 87.7±15 % maximum relaxation; EC_50 118 mM, 95% CI 77–178 mM; n=18) compared to control rats (E_max 96.0±0.9 %, EC_50 53 mM, 41–68 mM, n=17). Inhibition of the nitric oxide (NO) pathway using L-nitro-arginine methyl ester (L-NAME, 100 μM) and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 1 μM), did not significantly change ACh responses (ANOVA, P<0.05) in either group of rats. Remaining relaxations were blocked by the addition of the small- and intermediate/large-conductance Ca^{2+}-activated K^+ channel inhibitors, apamin (1 μM) and charybotoxin (0.1 μM), respectively. Inhibition of the EDHF pathway with apamin (1 μM) together with the intermediate-conductance Ca^{2+}-activated K^+ channel inhibitor, TRAM-34 (1 μM), significantly impaired (ANOVA, P<0.05) ACh-mediated relaxations in both diabetic and control rats. The residual response was abolished by the addition of the large-conductance Ca^{2+}-activated K^+ channel inhibitoriberiotoxin (0.1 μM). The present study indicates that in type-1 diabetes, there is endothelial dysfunction in cremaster arteries. The findings also suggest that ACh-mediated relaxations are mediated by EDHF, and involve small-, intermediate- and large-conductance Ca^{2+}-activated K^+ channels.

**FENOFIBRATE EXAGGERATED ACTH-INDUCED HYPERTENSION IN RATS**

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In the present study, we investigated the effect of fenofibrate on glucocorticoid-induced hypertension in rats. Male Sprague-Dawley rats were treated with saline, adrenocorticotropic hormone (ACTH) (0.2 mg/kg/day) or dexamethasone (109 μg/kg/day) s.c. for 13 days. Fenofibrate (100 mg/kg/day per rat) was mixed in ground food. Systolic blood pressure (SBP) was increased by ACTH (108 mg/kg/day for 13 days) and dexamethasone increased renal microsome 20-HETE formation and thymus weight. ACTH and dexamethasone increased renal microsome 20-HETE formation and thymus weight. ACTH increased aortic superoxide production while dexamethasone decreased plasma NO3 concentrations or aortic superoxide production. The expression of CYP2C23, CYP2C11 or CYP4A was not affected by fenofibrate, ACTH or dexamethasone. In conclusion, fenofibrate exaggerated ACTH- but not dexamethasone-induced hypertension. The effect of fenofibrate on ACTH-induced hypertension was independent of 20-HETE production.

**ANALYSIS OF BIOCHEMICAL CHANGES IN THE HEARTS OF ADULT INTRAUTERINE GROWTH RESTRICTED OFFSPRING USING FTIR IMAGING MICROSPETOSCOPY**

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Growth restriction in utero as a result of maternal malnutrition has been linked with gender specific decline in cardiac performance during adulthood. Here we examined the effect of growth restriction in rats, due to maternal low protein diet (LPD), on the changes in fibrosis and biochemical composition of the heart in the female offspring in adulthood. Wistar Kyoto (WKY) dams were administered either LPD (6.7% casein) during pregnancy and lactation or normal protein diet (NPD; 20% casein). At 14 weeks of age, hypertension was induced through a 4 week continuous infusion of angiotensin II (ANGII 200ng/kg/min) in female NPD and LPD offspring (N=7) via subcutaneous minipumps while control group received saline. Arterial blood pressure was measured using a tail cuff method. At 18 weeks of age the offspring were perfusion fixed and organs collected. The hearts were weighed, sliced and the heart volumes stereologically determined according to the Cavalieri method. Alternate left ventricle (LV) slices from normotensive and hypertensive offspring were used for assessment with Fourier transform infrared (FTIR) imaging micro-spectroscopy. FTIR images were processed using Unsupervised Hierarchical Cluster Analysis. Birth weights of the LPD offspring were significantly lower compared to NPD (6.5±0.3 g and 7.6±0.3 g, respectively), however in adulthood (18 weeks of age) this difference was no longer significant. ANGII infusion resulted in lower body weight in LPD and LPD offspring (P<0.0002). Absolute heart and LV volumes were both significantly lower in LPD offspring. Relative heart and LV volumes were significantly increased by ANGII administration. Perivascular fibrosis and both media to lumen and adventitia to lumen ratios were increased in the NPD hearts only (P<0.009 and P<0.04, respectively). FTIR images recorded from the LV indicated significant differences in collagen distribution and density between NPD and LPD hearts that is attributed to collagen disorder in the LPD hearts. The LPD hearts had lower intensity amide-I band but overall higher optical density in the mid infrared. FTIR imaging spectroscopy shows promise as an independent modality for examining changes in the macromolecular chemistry of the adult IUGR heart.
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