

HIGHLIGHT ON: Proceedings of the 11th Annual Symposium of the London Hypertension Society

New themes in hypertension

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Introduction

The London Hypertension Society provides a forum for basic scientists, clinical researchers, epidemiologists and public health experts to present and discuss results of their research into cardiovascular disease prevention and treatment. The Society holds evening scientific meetings at the Medical Society of London five times a year during academic terms with speakers on research from throughout the United Kingdom as well as visiting international speakers. In addition, annual symposia and theme workshops on aspects of hypertension research have been held by the Society since 1990 with publication of proceedings of the annual symposia since the 1991 2nd Annual Meeting (1). The abstracts published in this issue of *Nutrition, Metabolism and Cardiovascular Disease* are based on peer review presentations from the 11th Annual Symposium of the London Hypertension Society held at the Royal College of Surgeons in London on 18th April 2000. At this meeting our invited lecturers discussed progress in basic research and in the prevention and management of hypertension and its complications. Topics included genetics of salt sensitivity (Daniele Cusi, Milan, Italy) and of the angiotensinogen gene (Patricia Munroe, London).

Jacqueline Ohanian (Manchester) discussed the importance of diacyl glycerol kinase and other second messenger pathways in arteries. John Fuller (London) discussed management of hypertension in diabetes mellitus and Peter Whincup (London) reviewed approaches to defining and reducing cardiovascular risk in childhood. The importance of sex hormones in cardiovascular disease was discussed by Kay-Tee Khaw (Cambridge) and Alison Halliday (London) reviewed recent and new trials of treatment for patients with asymptomatic carotid artery stenosis. The prize for the best poster during the meeting was awarded to Dr LS Harrington (London) for her work on phospholipase C isoforms in human vascular smooth muscle cells.

The Web pages of the London Hypertension Society include information about evening meetings, future workshops and the 12th Annual Symposium of the London Hypertension Society to be held at the Royal College of Surgeons on Tuesday May 8th, 2001, along with details of how to become a member (<http://www.med.ic.ac.uk/dh/dhbm/lhs>)

Acknowledgements

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Reference

1. The role of circulating and local factors in hypertension (1992) Proceedings of the 2nd Annual Symposium of the London Hypertension Society. Eur J Intern Med 251-287.s

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The effect of changing dietary sodium intake upon red blood cell viscosity

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Background. A high sodium intake is associated with a higher risk of stroke, which is not completely explained by the increase in blood pressure that it causes [Antonios, MacGregor (1997) *J Hum Hypertens* 9: 511-515]. Several studies have suggested that a higher risk of stroke is also associated with a raised red blood cell (RBC) viscosity [Dormandy (1983) *Eur. Neurol.* 22 Suppl. 1: 23-29]. This suggests that a high sodium intake might increase stroke risk, independently of blood pressure, by increasing RBC viscosity. The aim of this study was, therefore to determine the effect of high and low dietary sodium intakes upon RBC viscosity.

Methods. Fifteen subjects were studied (10 male: 9 White; 6 Black). The study followed a randomised crossover double-blind protocol comparing the effects of 4-week high (200 mmol per day) and 4-week low (80 mmol per day) sodium diets. RBC viscosity was measured at the beginning of the study and at the end of each 4-week diet using solutions of isolated RBCs over a range of concentrations. The viscosity at 37°C was measured at a number of shear rates using a LS30 viscometer (Contraves) and the results were used to calculate the viscosity of a 55% RBC solution at a shear rate of 1s⁻¹.

Results. Urinary sodium excretion was significantly higher after 4 weeks on the high sodium intake compared with the low (84±32 vs 220 ± 74 mmol/24h; p<0.001) and there were also significant increases in both systolic (148 ± 18 vs 158 ± 19 mmHg; p=0.019) and diastolic (92 ± 10 vs 98 ± 8 mmHg; p= 0.005) blood pressure. However, there was no significant increase in RBC viscosity on the high sodium intake (13.14±2.27 vs 13.45±2.28 mPa.s; p= 0.512). This was also true when the White and Black subjects were analysed separately (p=0.662 and 0.649 respectively). The power of this study to detect a difference of 10% in RBC viscosity was 78%. The change in RBC viscosity between the high and low salt diets was also not correlated with the changes in urinary sodium excretion or the changes in blood pressure (p>0.18 in all cases).

Conclusions. These results are the first demonstration in human subjects that a high sodium intake does not raise the viscosity of isolated washed RBCs, at least over a 4-week period. It therefore seems unlikely that the stroke risk of a high sodium diet is associated with increases in RBC viscosity.

Atypical antagonism of human ET_A receptors by BQ123 and FR137319: potential role of alternative splicing of the ET_A-receptor gene

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Background. There is increasing evidence that the receptors that mediate the different actions of endothelin (ET) in human blood vessels do not fit the present receptor classification. We have previously shown that the atypical effects of low concentrations of the ET_A receptor antagonist BQ123 are not affected by inhibition of endothelium-derived vasoactive factors, endothelial removal and ET_B receptor desensitisation (Pate et al., *J. Cardiovasc. Pharmacol.* 1999; 33: 567-572).

Methods and Results. Using samples of human saphenous vein set-up in traditional organ baths, we have observed that increasing concentrations (10⁻⁶M, 3x10⁻⁶M & 10⁻⁵M) of the ET_A receptor antagonist BQ123 (n=4) produces non-parallel rightward shifts of the ET-1 concentration-response curve in samples of human saphenous vein. A similar pattern of antagonism can also be seen with the same range of concentrations of another ET_A receptor antagonist FR137319 (n=4). BQ123 (10⁻⁶M) and FR137319 (3x10⁻⁶M) only block the effects of low concentrations of ET, but high concentrations then shift the upper portion of the ET concentration-response curve. RT-PCR analysis of hET-AR gene expression in saphenous vein samples shows the presence of at least three different transcripts – one of the predicted size, and two smaller products corresponding to in-frame deletions of exons 3 and 4, and exon 3 of the hET-AR gene. Furthermore, we have shown that additional hET-AR gene transcripts are expressed, in a subset of patient samples that are also subject to alternative mRNA splicing, providing evidence that a mixed population of hET-AR gene transcripts exists in the human vasculature.

Conclusions. These data suggest that alternative splicing of the human hET-AR gene may give rise to functionally distinct ET_A receptor subtypes.

The effect of modest longer-term salt reduction on blood pressure. A meta-analysis of randomised controlled trials. Implications for public health

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Background. There have been several meta-analyses of the effect of reducing salt intake on blood pressure. But these meta-analyses either included trials with a very short duration e.g. 5 days, and/ or included severe reductions of salt intake to less than one gram (10-20 mmol) per day. These studies therefore can not be used to refute public health recommendations for a longer term and more modest reduction in salt intake.

Objective. We carried out a meta-analysis of modest salt reduction excluding trials of less than 4 weeks duration.

Methods: The inclusion criteria were 1) random allocation to experimental conditions; 2) no concomitant interventions in either groups; 3) reduction of 24 hr urinary sodium ≥ 40 mmol/day and excluding trials where 24 hr urinary sodium excretion was less than 30 mmol; 4) study duration ≥ 4 weeks. The analyses were weighed by the inverse variance.

Results. Twenty-six trials with 2762 subjects were found that fitted the above criteria. Sixteen trials were in hypertensives ($n=658$, median age: 49 years, median duration: 6 weeks) and 10 trials in normotensives ($n=658$, median age: 49 years, median duration: 4 weeks). The reduction in 24 hr urinary sodium was 78 mmol (53-117 mmol) in hypertensives and 74 mmol (40-118 mmol) in normotensives. The blood pressure fell by $4.2/2.4 \pm 0.4/0.3$ mmHg in hypertensives ($p < 0.001$ for both SBP and DBP) and by $1.6/0.6 \pm 0.3/0.2$ mmHg in normotensives (SBP: $p < 0.001$; DBP: $p < 0.05$). Weighted linear regression analyses showed dose response relationship between the change in urinary sodium and blood pressure. A reduction of 100 mmol/day in salt intake predicts a fall in blood pressure of $6.1/3.5$ mmHg in hypertensives and $2.7/0.9$ mmHg in normotensives.

Conclusions. Our results demonstrate that a modest reduction in salt intake for at least 4 weeks does have a significant effect on blood pressure in both normotensive and hypertensive subjects. These therefore support other evidence for a reduction in population salt intake.

PLC isoforms are associated with the differentiation status in human vascular smooth muscle cells

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Background. Phospholipase C (PLC) is important in signal transduction linking a wide range of extracellular signals to intracellular responses via the hydrolysis of

Phosphoinositide 4,5-bisphosphate (PIP_2). The products of this reaction act as key second messengers in a number of signaling pathways, including those leading to smooth muscle growth and contraction. The members of the Phospholipase C family of enzymes are differentially regulated and expressed and this is accepted as the mechanism by which different cell stimuli access this biochemical event. A wider implication however, may be differential cellular function.

$PLC_{\gamma 1}$ is stimulated by growth factors and is highly expressed in foetal tissue and colon carcinoma cell lines, suggesting that this isoform may be involved in cell division and growth. $PLC_{\gamma 1}$ expression is also upregulated in vascular smooth muscle cells (VSMC) as they dedifferentiate and take on a synthetic phenotype. In contrast, $PLC_{\delta 1}$ has not been linked to growth, but has been identified as the predominant isoform of PLC in differentiated contractile VSMC. $PLC_{\delta 1}$ has also been linked to the monomeric GTPase rhoA which acts as an inhibitory modulator of $PLC_{\delta 1}$ activity. Thus providing possible a link between $PLC_{\delta 1}$ and cytoskeletal organisation.

Methods and Results. Human saphenous vein derived VSMC were cultured using an explant technique. The PLC isoform profile of randomly cycling cells was characterized by Western blots. Although PLC β_1 , β_3 , β_4 , $\delta 1$, $\delta 2$, $\gamma 1$, $\gamma 2$ were all present in rat brain, only $PLC_{\delta 1}$ and $PLC_{\gamma 1}$ were present in the VSMC. The effect of differentiation status on the relative expression of these isoforms as well as SM α -actin was investigated using the following methods: 1) Serum withdrawal for seven days; 2) $10 \mu M$ Retinoic Acid for 72 hours (replaced every 24 hours); 3) $3 \mu M$ lovastatin for 24 hours. Protein expression was measured by densitometric analysis. The results show the mean \pm SEM of the percentage change in protein expression.

	SM α -actin	PLCd1	PLC γ 1	n=
Serum Withdrawal	290 \pm 243**	247 \pm 167*	-46 \pm 6***	7
Retinoic Acid	83 \pm 27*	232 \pm 94*	-62 \pm 13*	5
Lovastatin	76 \pm 23*	347 \pm 283**	-48 \pm 10*	8

*= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$ by Student's T test:

Conclusions. All three methods induced a measure of re-differentiation of the VSMC as indicated by a significant increase in SM α -actin expression. The expression of $PLC_{\delta 1}$ and $PLC_{\gamma 1}$ appear to be inversely related to each other, with an increase in $PLC_{\delta 1}$ expression being associated with an increase in SM α -actin. The nature of the PLC isoform profile coupled with the absence of PLC β isoforms may have a

profound influence on the accepted roles of PLC isoforms in VSMC.

Mitochondrial DNA deletions in fibrillating atria

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Background. Mitochondrial DNA (mtDNA) deletions have been associated with several cardiac disorders including cardiomyopathy. Persistent tachycardia (including atrial fibrillation, AF) has been documented in animal models to cause ventricular myopathy. Significant mtDNA deletion may therefore result in AF. The aim of this study was to investigate mtDNA deletion in AF patients.

Method. Right atrial appendage (RAA) biopsies were harvested from 15 patients with AF and 12 age-matched control patients in sinus rhythm, undergoing elective cardiac surgery. All samples were cut into 5–15 slices (5–20 mg each) and total DNA was extracted by standard methods. The presence of deletions in mitochondrial DNA in all samples was investigated with the use of standard and long range polymerase chain reaction (PCR) based on methods published by Zhang et al, 1992 and Paul et al, 1996. All investigations were evaluated by two people who were blind to the disease entity being investigated and the clinical characteristics.

Results. A large number of mtDNA deletions were found in all tissue samples from AF patients and controls. Their size ranged widely (500 bp to several kb) depending on the primer pairs used for PCR amplification.

Size (kb)	Controls Slices (No)	AF patients Slices (No)
0 - 1.0	29	33
1.0 - 2.0	52	60
2.0 - 3.0	10	10

Conclusions. Multiple deletions of mtDNA were detected in atrial appendage samples from AF patients and controls. However no apparent association was found between the presence of mtDNA deletions and AF. It is believed that mtDNA deletions accumulate during the process of normal ageing and therefore their presence may not necessarily be of pathogenic significance.

Changes in dietary sodium intake are associated with change in membrane fluidity in individuals of Afro-Caribbean origin

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Background. Afro-Caribbeans have a high incidence of hypertension, which is often characterized by a greater blood pressure response to changes in dietary sodium intake. Abnormalities in intracellular transport mechanisms may be important in the development of hypertension and the response to sodium intake in these individuals. Membrane transport processes are affected by the nature and composition of the membranes, which in turn may be affected by serum lipid levels. Changes in cellular membrane fluidity, an index of the membrane properties, have been demonstrated in essential hypertension and have been associated with changes in ion-transport activity.

Methods. The purpose of this study was to investigate the effect of changes in dietary sodium intake on membrane fluidity using the fluorescent probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and trimethylammonium DPH (TMA-DPH). Erythrocyte membranes were isolated from 10 individuals of Afro-Caribbean descent with a mean blood pressure of 159.6±20.6 / 95.0±8.3 mmHg on their normal sodium intake. Each individual was studied on their normal diet and also after 4 weeks on both a low (60-80 mmol/24 h) and high (180-200 mmol/24 h) salt diet in a randomized, double-blind, crossover study.

Results. The results of the study are given in the Table below (Values are means ±SD).

	Low salt	High salt	p
Lying systolic BP (mmHg)	154.0±18.6	159.1±17.9	0.047
Lying diastolic BP (mmHg)	92.9±8.6	98.3±11.3	0.009
Serum cholesterol (mmol/L)	5.3±0.7	5.2±0.8	0.25
Serum triglycerides (mmol/L)	1.04±0.41	0.99±0.49	0.51
Urinary sodium (mmol/24 h)	69.3±21.5	172.2±28.7	<0.001
DPH	0.220±0.005	0.219±0.007	0.53
TMA-DPH (n=9)	0.260±0.004	0.256±0.005	0.027

The high salt diet was associated with a significant increase in blood pressure and a significant increase in membrane fluidity as measured by TMA-DPH but not DPH. However, there was no association between the change in blood pressure and the change in fluidity. There were also no significant changes in serum lipid levels.

Conclusions. The results of this preliminary study suggest that changes in dietary sodium intake may affect the properties of erythrocyte membranes in individuals of Afro-Caribbean origin. These changes are apparently independent of serum lipids and appear to be confined to the outer regions of the membrane bilayer. However, the possible significance of these changes in relation to changes in blood pressure remains to be determined.

Vitamin C and endothelium dependent-dilatation in healthy elderly subjects

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Background. Age is an independent risk factor for cardiovascular disease. The endothelium releases nitric oxide (NO) which dilates resistance vessels. Ageing is associated both with an increase in oxidative stress and a progressive decline in endothelium-dependent vasodilatation. The anti-oxidant vitamin C has been used to augment NO-mediated dilatation in vivo in subjects with classical cardiovascular risk factors. We assessed the effect of vitamin C on endothelium-dependent dilatation in healthy elderly subjects.

Methods. Bilateral venous occlusion plethysmography was used to measure forearm blood flow (ml/min/100 ml forearm volume) during intra-arterial infusion of the endothelium-dependent dilator acetylcholine (ACh) and the NO donor glyceryl trinitrate (GTN) in 16 healthy subjects, mean age 66 (range 57-79 years), 11 female, all Caucasian, BP123/75 ± 4/3(SEM)mmHg. Responses to ACh (25, 50 and 100 nmol/min) or GTN (4, 8 and 16 nmol/min), each dose for 3 min, were determined alone, then during vitamin C (25 mg/min) infusion. Effects of treatment were calculated from ratio of blood flow in infused to control arm, expressed as percentage (±SEM). Dose-response curves compared by AUC analysis using Wilcoxon's sign rank test.

Results. ACh and GTN caused dose-dependent dilatation of the forearm resistance bed. At the highest doses of ACh and GTN, FBF increased by 399±48% and 127±17% respectively. Vitamin C had no significant effect on either

basal blood flow or the dilatation caused by ACh (1820±226 before; 1724±204 during vitamin C; $p=0.63$) or GTN (AUC 543±75 before; 574±91 during vitamin C; $p=0.75$).

Conclusions. These results suggest vitamin C does not acutely increase the bio-availability of endogenous or exogenous NO in healthy old age and are consistent with functionally normal vascular anti-oxidant defences in this group.

Platelet sodium-hydrogen exchanger activity and left ventricular mass

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Background. The sodium-hydrogen exchanger (NHE) is integral to the processes that facilitate cell growth. Several studies have reported that the activity of the exchanger may be elevated in individuals with essential hypertension. The aim of this study was to examine the potential contribution of exchanger activity to the development of left ventricular hypertrophy (LVH) by investigating the relationship between platelet sodium-hydrogen exchanger activity and left ventricular mass.

Methods. Twenty male Caucasian subjects (mean age±SD: 48±13; body mass index: 29±4kg/m²) with a wide range of blood pressures were studied (mean BP: 152+22/93±15 mmHg; range: 115-190/61-117 mmHg). All subjects had not taken any medication for the previous 4 weeks and were on their usual dietary sodium intake. Sodium-hydrogen exchanger activity in isolated platelets was determined by measuring the rate of recovery in intracellular pH from nigericin-induced acidosis, using the fluorescent dye BCECF. Left ventricular mass (LVH) was measured by M-mode echocardiography and used to calculate left mass index (LVMI). Analysis of association between continuous variables were examined using parametric correlation analysis.

Results. The mean rate of pHi recovery was 0.15±0.03 dpHi/s (range: 0.09-0.21). Mean LVM and LVMI were 245±70 g (range: 127-367) and 120±32 g/m² (range: 56-178) respectively. LVMI was not significantly correlated with either systolic ($r=0.39$, $p=0.09$) or diastolic blood pressure ($r=0.27$, $p=0.3$). Platelet sodium-hydrogen exchanger activity was not significantly correlated with either left ventricular mass ($r=0.13$; $p=0.6$) or left ventricular mass index ($r=0.06$; $p=0.8$). Seven subjects were noted to have left ventricular hypertrophy (LVM>133g/m²). Platelet sodium-hydrogen

exchanger activity was not significantly different between subjects with and without left ventricular hypertrophy.

Conclusions. *The results of this study show that platelet sodium-hydrogen exchanger activity is not significantly correlated with either left ventricular mass or left ventricular mass index. These data contrast with previous findings showing that left ventricular mass is correlated with exchanger activity in leucocytes and erythrocytes.*

Regional differences in endothelial-dependent relaxant function: effects of nitric oxide, high glucose and oxidant stress

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Background. *Hypertension and other cardiovascular complications are common in diabetic patients. We have previously reported regional differences both in basal endothelial-dependent relaxant function and in endothelial dysfunction induced acutely by high glucose. We assess here the role of nitric oxide and redox stress in these regional differences.*

Methods. *Rings of thoracic aorta (TA) and carotid artery (CA) from 6 NZ white rabbits were incubated in organ baths for 6 hr in 5.5mM (NG) ± the nitric oxide (NO) syn-*

thase inhibitor L-NAME or 44mM glucose (HG) ± the superoxide scavenger superoxide dismutase (SOD). Concentration-responses to acetylcholine (ACh) were assessed after 2, 4 and 6 hr in rings pre-constricted with phenylephrine.

Results. *Relaxation to ACh was impaired in both TA and CA by 2, 4 and 6 hr of incubation in HG (ANOVA $F=6.53$, $p=0.01$). L-NAME decreased maximal relaxation to ACh from $60\pm 3\%$ to $9\pm 2\%$ for TA incubated in NG ($F=243$, $p<0.0001$) with no differences in TA vs CA. SOD altered endothelial dysfunction caused by HG ($F=9.45$, $p=0.0007$). In the carotid, SOD prevented endothelial dysfunction after 4 hr ($F=10.5$, $p=0.009$) but not after 6 hr incubation in HG. In contrast, in TA SOD did not prevent endothelial dysfunction after 4 hr HG, and after 6 hr incubation endothelial dysfunction in TA was worse for HG combined with SOD than for HG alone ($F=2.9$, $p=0.014$).*

Conclusions. *Differences in basal NO release do not directly explain regional differences in vascular function. Our results support a complex role for superoxide in regional differences in HG-induced ED, with superoxide mediating early HG-induced ED in carotid artery but protecting from later HG-induced ED in thoracic aorta. These findings may help to explain regional differences with other pathology such as hypertension in which oxidative stress and endothelial dysfunction are candidate mechanisms for increased cardiovascular risk.*