1.29±0.35 IU/ml, p=0.006; fibrinogen 2.97±0.7 g/l to 2.77±0.38 g/l, p=0.07; vWF, 1.15±0.35 IU/ml to 1.07±0.28 IU/ml, p=0.003 and AT-III, 107.9±16.0% to 103.3±13.8%, p=0.04. There was no significant change in FVII:C levels. There was a significant linear association after 3 months between changes in HbA_{1c} and FVIII:C, r=0.71, p<0.001; fibrinogen, r=0.72, p<0.001 and vWF, r=0.47, p<0.001. These results indicate that metabolic control is related to FVIII:C, vWF and fibrinogen levels and that abnormalities of haemostasis may provide a link between glycaemic control and vascular disease.

8 PC 10 IMMUNOSTAINING OF HUMAN PITUITARY ADENOMAS IN VITRO MAY BE AN UNRELIABLE MARKER OF PROLIFERATION

Atkin SL*, Landolt AM+, Jeffreys RV#, Foy P# and White MC*.

*University Department of Medicine, PO Box 147, Liverpool, UK. #Neurosurgical Unit, Walton Hospital, UK, and +Zurich, Switzerland.

The labelling index in 9 human anterior pituitary adenomas (6 GH, 2 FSH/LH and 1 LH) was determined after 4 days in serum free medium employing monoclonal antibodies against PC 10 and bromodeoxyuridine (BDU) labelling. The monoclonal antibody PC 10 has a strong affinity for proliferating cell nuclear antigen (PCNA), an auxiliary factor to DNA polymerase δ. PCNA has been used as a marker of proliferation being detected throughout the cell cycle from G_1, unlike BDU which is specific for the S phase. Human pituitary adenomas were dispersed to single cells and plated out at 10^5 cells/ml/ in 24 well plates in Iscoves modified Dulbecco's medium (IMDM) supplemented with 10% foetal calf serum and allowed to attach. Cells were washed and the medium replaced with serum free IMDM. After 4 days in situ viability was assessed using a graticule and fluorescence and 10^{-5}M bromodeoxyuridine was added to the adenoma cells for one hour before fixing in methanol at -20°C. Duplicate wells were then immunostained with either anti-BDU or PC 10 and double stained with neuron specific enolase to identify proliferation and the adenoma cells respectively. The labelling index (LI) was the number of proliferating cells per 1000 pituitary cells studied.

Two tumours were negative for both BDU and PCNA staining, 2 tumours were negative for BDU but positive for PCNA staining (LI 0.8% and 3.6%) and 1 tumour gave comparable results (LI 0.1% BDU, 0.2% PCNA). Marked positivity in 4 tumours (3 GH + 1 LH) was found with PCNA (LI 9.4%, 47.5%, 80%, 87%) compared to BDU (LI 0.3%, 1.4%, 1%, 1%) respectively.

Antigen cross reactivity is the most likely explanation for the very high PCNA labelling index compared to that found with BDU. We conclude that the monoclonal antibody PC 10 against PCNA is a less reliable marker for proliferation than the use of BDU in this in vitro system.

9 EXAGGERATED VASCULAR SENSITIVITY TO NORADRENALINE: A CONTRIBUTORY FACTOR TO HYPERTENSION IN PHAEOCHROMOCYTOMA

CW BODMER, MW SAVAGE (introduced), MA WARE (introduced), and G WILLIAMS

University Department of Medicine, PO Box 147, Liverpool L69 3BX, England

A 31-year old man presented with a 6-month history of night sweats; high blood pressure had been found on one occasion two years previously but not followed up. Blood pressure at presentation was 154/112 mm Hg. Phaeochromocytoma was diagnosed by grossly elevated urinary noradrenaline excretion (27,370 nmol/24h; normal range 120-590), with normal adrenaline and dopamine. Neuropeptide Y, a powerful vasoconstrictor, is secreted by some phaeochromocytomas but plasma levels were normal in our patient. MIBG and abdominal CT scans indicated two large para-aortic phaeochromocytomas, which were successfully removed at surgery, with resolution of the symptoms and hypertension. We examined the vasoconstrictor responses to locally-infused noradrenaline (NA) before and after surgery. A dose-response curve was constructed using a stepped infusion of NA (1-128 ng/min) into a dorsal hand vein using an established technique, and the dose causing 50% of maximal vasoconstriction (ED_{50}) was calculated. The patient was hyper-responsive to NA, showing a significant left-shift of his dose-response curve, with an ED_{50} of 3.6 ng/min (range for 20 normal subjects 6.0-157.8). Sensitivity was normal following tumour removal (ED_{50} 15.9 ng/min). We suggest that hypersensitivity to the vasoconstrictor effects of NA may exacerbate hypertension in phaeochromocytoma. However, the mechanism is unknown.

10 IS INSULIN A SATIETY SIGNAL? INSULIN TREATMENT ANTAGONISES STARVATION-INDUCED INCREASES IN NEUROPEPTIDE Y CONCENTRATIONS IN THE ARCUATE NUCLEUS OF THE RAT HYPOTHALAMUS

UH MALABU, HD McCARTHY, PE McKIBBIN, C BURROWS AND G WILLIAMS

Department of Medicine, University of Liverpool, Liverpool L69 3BX

Neuropeptide Y (NPY), the most powerful appetite stimulant known, is synthesised in hypothalamic arcuate nucleus (ARC). NPY levels rise in the ARC and in appetite-regulating hypothalamic nuclei in food-deprived rats, and may drive compensatory hyperphagia in starvation. *Circulating insulin levels fall in starvation and insulin deficiency has been* postulated to stimulate hypothalamic NPY; this supports the suggestion that insulin acts on the brain to inhibit feeding. We tested this hypothesis by determining whether the increase in NPY in the ARC of starved rats was suppressed by insulin treatment.

Adult male Wistar rats were studied. Controls (n=8) were freely-fed and two other groups were food-deprived for 72 hours, both losing 20% of initial weight (p<0.001 vs controls). One food-deprived group (n=10) recieved insulin (5 U/kg/day) injected subcutaneously twice daily and both other groups recieved saline. Mean blood glucose values (measured in tail-prick samples) were 5.9 ± 0.1 mmol/l in controls, 4.6 ± 0.3 mmol/l in food-deprived (p<0.001, vs controls) and in insulin-treated 4.4 ± 0.3 mmol/l (p<0.001 vs controls; NS vs food-deprived group). Final plasma insulin levels in insulin-treated rats were higher than in saline-treated food-deprived rats (46.6 ± 8.9 vs 28.9 ± 4.5 pmol/l; p<0.001) and comparable with controls (52.6 ± 16.2 pmol/l; p=NS). ARC NPY concentrations rose significantly above controls in food-deprived rats (14.18 ± 1.79 vs 8.4 ± 2.16 fmol/ug protein; p<0.001) and were *intermediate in the insulin-treated food-deprived* group (11.19 ± 1.36 fmol/ug protein: p<0.01 vs controls and p<0.001 vs saline-treated, food deprived). Other hypothalamic regions showed no differences between groups.

Insulin therefore antagonises fasting-induced increases in NPY concentrations in the ARC. This is consistent with the hypotheses that insulin deficiency stimulates hypothalamic NPY synthesis, and that peripheral insulin acts as a satiety factor by inhibiting hypothalamic NPY.

11 D-FENFLURAMINE (D-FEN) IMPROVES INSULIN SENSITIVITY AND GLUCOSE TOLERANCE IN INSULIN-DEFICIENT RATS

*PE McKIBBIN, *HD McCARTHY, *U MALABU, *S DRYDEN, *A KILPATRICK and WILLIAMS G *intro

Department of Medicine, Royal Liverpool University Hospital, Liverpool L69 3BX, England

D-Fen, a widely used anorectic drug, is reported to improve insulin sensitivity and glycaemic control in human non-insulin dependent diabetes independently of its anorectic effect. A dose range of 0.5, 1.0, 2.5, 5.0 mg/kg of D-fen was given by gavage to male Wistar rats (n=10) that had been made diabetic by streptozotocin (55 mg/kg) for 3