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NF-kB activation in pulmonary inflammatory cells from premature infants with respiratory distress syndrome. **F C Cheah[1,2], C C Winterbourn[1], B A Darlow[2], M C M Vissers[1]. [1]Free Radical Research Group, Department of Pathology; [2]Department of Paediatrics, Christchurch School of Medicine and Health Sciences, University of Otago, Christchurch.**

Respiratory distress syndrome (RDS) is the most common pulmonary disorder affecting premature infants. Progression of RDS to chronic lung disease (CLD) of prematurity has been described as the neonatal pulmonary injury sequence. Inflammatory and oxidative damage and the lack of lung defence capacity, are the major factors involved. As activation of the cellular transcription factor, nuclear factor kappa-B (NF-κB), has been implicated in amplification of the inflammatory process in lung injury, we have investigated whether NF-κB activation occurs in premature infants with RDS.

Tracheal aspirate samples from mechanically ventilated infants were collected and the cells separated to be fixed for immunocytochemistry. Using an antibody targeting the p65 subunit of NF-κB and counter-staining this with anti-IgG-Cy3 antibody, the activation state of NF-κB was determined by its location in the cell; cytoplasm (inactive NF-κB), or nucleus (activated NF-κB).

The median gestation and birth weight of 20 premature infants who provided 58 tracheal aspirate samples, were 27 weeks and 795 g respectively. Fourteen infants (70%) had samples containing cells that showed activated NF-κB. Neutrophils were the predominant cells showing this activation in the first week of life. Macrophages with activated NF-κB were mainly seen in later aspirate samples. Occasionally, groups of epithelial cells were present but none showed activated NF-κB. Five infants with *Ureaplasma urealyticum* in their tracheal aspirates also showed NF-κB activation, and in two the activation continued despite treatment with erythromycin. Two thirds of infants with aspirates showing NF-κB activation progressed to develop CLD.

NF-κB activation in pulmonary inflammatory cells of premature infants with RDS indicates that these cells could amplify the inflammation that occurs in the neonatal pulmonary injury sequence. Inhibiting NF-κB activation may potentially limit acute lung injury and prevent the progression to CLD.

Urocortin-1 adsorption: implications for dose administration. **M Whitteker[1], M E Davis[2], E J Begg[1], G Hammond[2], J Livesey[2], M G Nicholls[2], A M Richards[2], Timothy G Yandle[2]. [1]Department of Clinical Pharmacology, Christchurch Hospital; [2]Christchurch Cardioendocrine Research Group, Christchurch Hospital and Christchurch School of Medicine.**

The aim of this work was to determine if urocortin-1 (Ucn-1) adsorbs to apparatus used in infusion studies.

Urocortin-1 is a 40aa vasoactive peptide currently under investigation with infusion studies in sheep and man. Peptides such as insulin and adrenomedullin adsorb strongly to PVC and glass. Given this, Ucn-1 adsorption characteristics needed investigation to enable accurate calculation of infusion dose, pharmacokinetics and dose/response data.

"Bench" infusion studies of radiolabelled Ucn-1 alone (pilot studies), unlabelled Ucn-1 (for RIA), and mixed (labelled plus unlabelled) Ucn-1, were undertaken to mimic infusions in sheep and man. Samples were taken for analysis at stages along the infusate preparation phase and at timed intervals during the bench infusions.

There was 20–45% loss of labelled hormone by 6 minutes at the end of infusion apparatus from both labelled and mixed (labelled and unlabelled) Ucn-1 infusate. Delivery appeared stable over the subsequent 48 minutes. In preliminary unlabelled studies, delivery of hormone was also stable over that time but recovery was variable between studies. In separate experiments, loss onto test tubes was up to 70% by 24 hours, less with glass than PVC.

Few peptide infusion studies have taken apparatus loss into account when assessing dose/response or pharmacokinetics. Without this information, doses delivered are unknown and the dose/response conclusions potentially invalid. The adsorption characteristics of Ucn-1 appear different to those of insulin and adrenomedullin. We suggest all peptides should undergo adsorption studies prior to use in infusion studies or in clinical practice.

Regulation of the adrenocorticotropin (ACTH) response to arginine vasopressin (AVP): mechanisms of desensitisation and resensitisation. A M A Hassan, D R Mason. Department of Zoology, University of Canterbury, Christchurch.

Recently, we have shown that treatment of ovine anterior pituitary cells with AVP, a physiological regulator of ACTH secretion, results in reduced responsiveness to subsequent stimulation with AVP. This desensitisation is rapid and readily reversible, suggesting that it might be mediated by receptor phosphorylation. Recovery from such desensitisation is thought to involve receptor internalisation and subsequent dephosphorylation by protein phosphatases. This study was aimed at investigating involvement of these processes in resensitisation of the ACTH response to AVP.

Perifused dispersed ovine anterior pituitary cells were stimulated with a 5 min pulse of AVP (100 nM). The response to this pulse was reduced by 55.8 ± 2.6% ($n = 18$, $p < 0.01$) if it was immediately preceded by a 15 min pre-treatment with 10 nM AVP. When a recovery period of variable duration was allowed between the pre-treatment and the pulse, resensitisation occurred. Recovery from desensitisation was complete within 20 min. Inhibition of receptor internalisation by treatment with 0.25 mg/ml concanavalin A for 70 min prior to the AVP pulse, reduced the extent of desensitisation induced by AVP pre-treatment rather than affecting resensitisation. Treatment with 10 nM okadaic acid, an inhibitor of protein phosphatase 1 and 2A, had no effect on either resensitisation or desensitisation. Inhibition of protein phosphatase

2B (PP2B) with 1 μM FK506 decreased the rate of resensitisation. Complete recovery from desensitisation took 40 min.

These results suggest that desensitisation of the ACTH response to AVP requires receptor internalisation and that resensitisation is dependent upon PP2B-mediated receptor dephosphorylation.

Adaptation of saccade amplitude in Parkinson's disease. M R MacAskill[1,2], T J Anderson[1,2,3], R D Jones[1,2,4]. [1]Christchurch Movement Disorders and Brain Research Group; [2]Department of Medicine, Christchurch School of Medicine; [3]Department of Neurology, Christchurch Hospital; [4]Department of Medical Physics and Bioengineering, Christchurch Hospital.

The accuracy of saccades (fast eye movements) is maintained over time, an adaptive ability usually ascribed to the cerebellum. Adaptation might occur elsewhere in certain tasks, such as in the prefrontal cortex for memory-guided saccades. We hypothesised that adaptation of memory-guided saccades would be impaired in Parkinson's disease (PD), as basal ganglia dysfunction can disrupt the operation of the prefrontal cortex, while adaptation of visually-guided saccades would be preserved.

Adaptation was induced by consistently yet imperceptibly displacing targets as saccades were made toward them, causing artificial saccadic inaccuracy. 12 PD subjects (off medication) and 12 age-matched controls performed 245 visually- and memory-guided horizontal saccades in two separate sessions. An infrared eye tracker detected the saccade, during which the target was displaced by 12.5% of the size of the initial jump, either in the same (centrifugal) or the opposite (centripetal) direction. PD subjects made smaller visually-guided saccades than did controls ($F(1,20) = 9.10$, $p < 0.01$), yet both groups modified saccade size appropriately. PD memory-guided saccades were also smaller than those of controls ($F(1,19) = 5.93$, $p < 0.05$). While controls decreased (by 8.6%) or increased (by 4.1%) the size of these saccades appropriately, PD subjects decreased saccade size in response to both centripetal adaptation (by an excessive 18.3%) and centrifugal adaptation (by 3.5%).

PD subjects were less able to modify saccadic size appropriately when the movement size was specified in motor memory: a predilection for hypometria was invoked, regardless of adaptation direction. This indicates that in certain tasks adaptation may involve structures other than the cerebellum.