



## Proceedings of the 192<sup>nd</sup> Scientific Meeting of the Otago Medical School Research Society, Thursday 15 May 2008

### **Inhibitory action of taurine at GABA<sub>A</sub> and glycine receptors in the main olfactory bulb of the brain. K Igelstrom, P Heyward. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.**

Taurine is present in the brain at concentrations comparable to those of the major neurotransmitters GABA and glutamate, and can act at GABA or glycine receptors in several areas. Taurine is particularly abundant in the main olfactory bulb (MOB), but its role in this area is largely unknown. This study investigated the action of taurine on mitral cells (MC), the principal output neurons of the MOB.

Male Swiss outbred mice were decapitated and the MOB removed. Extracellular recordings were obtained from MC in horizontal MOB slices (350  $\mu$ m) maintained *in vitro* at 30°C. Taurine was bath-applied for 15 min, with or without receptor antagonists. The action potential frequency in the last 5 min of this period was compared with a 5 min recording made prior to drug application (paired *t*-test). Results are reported as a percentage change  $\pm$  SEM.

Taurine inhibited MC spontaneous firing at 0.5 and 1 mM ( $-8.55 \pm 2.39\%$ ,  $n = 6$ ,  $P < 0.01$ ; and  $-36.7 \pm 8.19\%$ ,  $n = 10$ ,  $P < 0.001$ , respectively). Inhibition by 1 mM taurine was unaffected by glycine receptor (GlyR) antagonist strychnine (1  $\mu$ M;  $-21.9 \pm 4.04\%$ ,  $n = 11$ ,  $P < 0.001$ ), and GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) antagonists bicuculline (50  $\mu$ M;  $-19.4 \pm 6.05\%$ ,  $n = 8$ ,  $P < 0.05$ ) and gabazine (10  $\mu$ M;  $-20.0 \pm 6.94\%$ ,  $n = 8$ ,  $P < 0.01$ ). However, the non-specific chloride channel blocker picrotoxin (0.1 mM) abolished taurinergic inhibition ( $-0.98 \pm 7.27\%$ ,  $n = 7$ ,  $P > 0.05$ ), as did simultaneous application of GlyR and GABA<sub>A</sub>R antagonists (bicuculline + strychnine,  $-0.52 \pm 7.49\%$ ,  $n = 4$ ,  $P > 0.05$ ; gabazine + strychnine,  $-4.65 \pm 5.18\%$ ,  $n = 6$ ,  $P > 0.05$ ).

These results suggest that taurine inhibits MC via chloride channels with unusual pharmacology. Further research is needed to understand the contribution of the GABA<sub>A</sub>R and GlyR.

### **Destabilised adhesion and c-Src activation characterise inherited lobular breast carcinoma from E-cadherin (CDH1) mutation carriers. HJ Kwon<sup>1</sup>, D Zou<sup>1</sup>, V Blair<sup>2</sup>, B Humar<sup>1</sup>. <sup>1</sup>Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin; <sup>2</sup>Department of Surgery, University of Auckland, Auckland.**

E-cadherin (CDH1) germline mutations predispose to both hereditary diffuse gastric cancer (HDGC) and hereditary lobular breast cancer (HLBC). Early HDGC stages develop following down-regulation of the cell-cell adhesion molecule E-cadherin, whilst progression to submucosal tissue involves an epithelial-mesenchymal transition that is paralleled by activation of the sarcoma cellular oncogene kinase (c-Src) and its downstream target protein, signal transducer and activator of transcription 3 (Stat3).

Similar events have been reported for sporadic diffuse gastric cancer and lobular breast cancer, however HLBC has not been studied at a molecular level. In this study, two cases of HLBC were pathobiologically characterised with the following aims; to demonstrate similarities between the development of sporadic and hereditary LBC, and to provide a first rationale for the use of c-Src and Stat3 inhibitors as potential chemotherapeutic agents in HLBC.

Paraffin-embedded tissue from mastectomies of two patients carrying a *CDH1* germline 1008G>T mutation was examined using immunohistochemistry and immunofluorescence. Reduced expression of E-cadherin was observed from earliest stages onwards (atypical hyperplasia and *in situ* carcinoma) and was accompanied by down-regulation of other proteins ( $\beta$ -catenin, p120, Lin-7) that participate with E-cadherin in the adherens junction complex. Markers of normal mammary cells (CK5, CK18) demonstrated that HLBC differentiates along the luminal epithelial lineage. Progression to invasive carcinoma correlated with increased activities of c-Src and Stat3, with invasive cells showing a mesenchymal-like phenotype as evidenced by vimentin staining.

This study provides the first pathobiological description of HLBC. Our results suggest HLBC develops similar to its sporadic counterpart with regards to the initiating event (down-regulation of adhesion), its differentiation path and further progression to invasive disease. Our observation that c-Src and Stat3 activities both correlate with invasiveness of HLBC encourages the evaluation of corresponding inhibitors for the treatment of this disease.

**An evidence-based approach to human dermatomes. M Lee, R McPhee, M Stringer. Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.**

A dermatome is the area of skin sensation supplied by one spinal nerve. It is a fundamental concept in human anatomy and of major importance in clinical diagnosis. Despite this, there are major discrepancies in current dermatome maps in standard anatomy and clinical texts. The aim of this study was to undertake a detailed systematic literature review of the evidence for the distribution of human dermatomes.

A thorough search of several electronic databases was conducted, together with a hand search of papers. Two independent observers analysed each paper to improve objectivity. Emphasis was placed on the technique of ascertainment, dermatome location and extent, number of subjects studied, and methodologic limitations of each study. Studies were graded into one of three categories using a scheme adapted from evidence-based clinical medicine: good (accurate methodology, further research unlikely to change the result, reasonable consistency in data, appropriate numbers of cases); intermediate (further research likely to change the result, deficiencies in methodology or sample size); or poor (very uncertain contribution).

Currently, the best available evidence is derived from mapping cutaneous sensory disturbances in humans by three methods of investigation: sectioning of adjacent dorsal nerve roots; Herpes zoster skin eruptions with histological confirmation of nerve root involvement; and recording of mixed spinal nerve sensory action potentials after electrical skin stimulation. Based on these findings, a novel evidence-based

dermatome map was constructed by a professional medical illustrator. This represents the most consistent tactile dermatomal areas associated with each spinal dorsal nerve root found in most individuals. The map not only highlights the orderly arrangement and areas of consistency of dermatomes, but also emphasizes overlap and variability.

In conclusion, this review demonstrates i) that current dermatome maps are inaccurate and based on flawed studies and ii) the validity of an evidence-based approach to an anatomical concept.

**Pyoverdine production by *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. M McNeil, I Lamont. Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin.**

The bacterial pathogen, *Pseudomonas aeruginosa*, is the major cause of chronic lung infections in cystic fibrosis patients. Iron is important for the survival of *P. aeruginosa*, but within hosts the levels of free iron are low. To overcome this *P. aeruginosa* produces an iron-chelating compound known as pyoverdine. Sputum samples from cystic fibrosis patients have high levels of pyoverdine, whilst pyoverdine deficient mutants have a reduced ability to cause infection in animal models of disease, emphasising the role of pyoverdine in infection. This study aimed to determine the amount of pyoverdine produced by clinical isolates of *P. aeruginosa* from cystic fibrosis patients and the molecular basis for variation between isolates.

Screens on agar plates were used to detect pyoverdine production, as when pyoverdine is produced *P. aeruginosa* emits a yellow-green fluorescence. The amount of pyoverdine produced by each isolate was quantified using a fluorescence assay. The expression of a key pyoverdine synthesis gene (*pvdE*) was then analysed in reporter assays.

Eighteen percent of clinical isolates (3/17) were pyoverdine deficient. There was also considerable variation in the amount of pyoverdine produced by the remaining isolates (62 – 1173  $\mu\text{mol}$ ) and all produced less than a well characterised laboratory strain (2061  $\mu\text{mol}$ ). Reporter assays identified that pyoverdine-deficient strains did not express PvdE. Pyoverdine-deficient strains were able to utilise pyoverdine when it was present in the environment, despite not being able to produce it.

The results from this study illustrate that there is considerable variation in the amount of pyoverdine produced by clinical isolates, with some isolates being pyoverdine deficient. Despite this, all clinical isolates utilise pyoverdine as a means of acquiring iron, making pyoverdine an important factor in the successful establishment of infections. These results support research investigating the use of pyoverdine as a potential drug target for *P. aeruginosa* infection.

**The expression of toll-like receptor 2 and toll-like receptor 4 in oral squamous cell carcinoma and irritative hyperplastic lesions. L Ng, A Rich, G Seymour. Department of Oral Diagnostic and Surgical Sciences, School of Dentistry, University of Otago, Dunedin.**

The toll-like receptors (TLRs) are transmembrane proteins expressed by chronic inflammatory cells (CIC) and endothelial cells (EC) during inflammation. TLRs induce reactive oxygen and nitrogen intermediates, initiate signal transduction cascades and activate apoptotic pathways. This study investigated TLR2 and TLR4 expression by CIC and EC in oral squamous cell carcinoma (OSCC) and irritative hyperplasia (IH) to determine the possibility of using TLRs as a marker of potential malignancy.

Thirty-two archival OSCC and 15 IH were stained via immunohistochemistry (primary antibodies TLR2: sc-21759 and TLR4: sc-8694, Santa Cruz Biotechnology, California, USA) and counterstained with haematoxylin and eosin. A minimum of 1000 cells in total (mixture of CIC and EC) per sample was systematically assessed with light microscopy and the proportion of positively stained cells to negatively stained cells determined. TLR expression was recorded as positive when there was crisp dark brown cellular staining.

TLR4 showed no positive staining. TLR2 expression in OSCC (mean = 14.1%, SD = 10.2%, n = 32) compared to IH (mean = 3.8%, SD = 7.5%, n = 15) was significantly higher (95% CI = 5.1 – 15.5). Standardisation for site between OSCC and IH confirmed the difference in TLR2 expression (alveolar ridge 95% CI = 3.0 – 20.0, lip 95% CI = 8.2 – 18.0, mucosa 95% CI = 2.4 – 15.0). Standardisation of variables showed OSCC from lip (n = 10) and tongue (n = 11) have significantly higher TLR2 expression (lip  $P < 0.02$ , tongue  $P < 0.01$ ). CI and  $P$  value calculation were based on the central limit theorem and standardised normal curve.

In conclusion, TLR2 expression is significantly higher in OSCC compared to IH. This supports the possibility of TLR being used as a marker of potential malignancy with potential therapeutic implications.

**Evaluating gentamicin and ototoxicity in neonates to optimise development of a new dosing regimen. K Owens<sup>1</sup>, C Sherwin<sup>2</sup>, D Reith<sup>2</sup>, N Medicott<sup>1</sup>. <sup>1</sup>School of Pharmacy and <sup>2</sup>Department of Paediatrics and Child Health, Dunedin School of Medicine, University of Otago, Dunedin.**

Gentamicin is a broad-spectrum aminoglycoside antibiotic that is often used in hospitals to treat neonates with suspected or confirmed sepsis. Neonates require relatively higher doses of gentamicin compared to adults due to their increased volume of distribution in proportion to their body size and decreased renal clearance. This increases the risk of ototoxicity, which can lead to permanent hearing impairment. The general incidence of hearing impairment in neonates is reportedly 4-5%.

A retrospective chart review was performed for 122 neonates treated with gentamicin in the Neonatal Intensive Care Unit (NICU) at Dunedin Hospital from September 2003 to November 2007. A clinical audit was undertaken to review hearing tests done

within 3–6 months of discharge on neonates who had received gentamicin treatment, which included prospective audiology data collected from NICU. Results were analysed by logistic regression using STATA® (version 9), with a measured outcome of hearing impairment. A one-compartment PK model was developed using NONMEM (version 5) to estimate the posthoc values of area under the curve (AUC) and maximum therapeutic concentration ( $C_{max}$ ).

It was found that the incidence of hearing impairment in the study population was 7.4%. The statistically significant independent variables associated with hearing impairment included total duration of treatment with all aminoglycosides (gentamicin and amikacin) (days) ( $P = 0.045$ ), gentamicin  $C_{max}$  (mg/L) ( $P = 0.009$ ), and gentamicin AUC (mg/L·h) ( $P = 0.005$ ). A logistic regression model was conducted, resulting in total duration of treatment with all aminoglycosides (days) as the most significant covariate ( $p$ -value of 0.005,  $R^2$  of 0.305).

The most significant variable associated with hearing impairment was total duration of aminoglycosides (days). This is the combination of total duration of gentamicin treatment (days) and total duration of amikacin treatment (days). These results will contribute to the development of a new dosing regimen for gentamicin in neonates.

**Recombinant sAPP $\alpha$  causes specific changes in gene expression in neuroblastoma cells and rat hippocampal cell slices. J Renshaw<sup>1</sup>, M Ryan<sup>2</sup>, J Williams<sup>2</sup>, W Tate<sup>1</sup>. <sup>1</sup>Department of Biochemistry, <sup>2</sup>Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.**

Alzheimer's Disease (AD) is a neurodegenerative disorder, marked by an increase in the soluble aggregate of amyloid beta ( $A\beta$ ), produced from internal cleavage of amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretase. APP can be cleaved alternately by  $\alpha$ -secretase producing secretory amyloid precursor protein alpha (sAPP $\alpha$ ). The endogenous levels of sAPP $\alpha$  and  $A\beta$  are kept in equilibrium by opposing activities of  $\alpha$ - and  $\beta$ -secretases. We propose that loss of sAPP $\alpha$  in diseased states contributes as much to cognitive decline as the accumulation of  $A\beta$ .

sAPP $\alpha$  is both neurotrophic and neuroprotective, but the biochemical mechanisms are unknown. This group has shown that sAPP $\alpha$  enhances specific gene expression in hippocampal cell slices, and stimulates *in vivo* long-term potentiation (LTP), the mammalian model for memory. The present study investigated gene expression in hippocampal cell slices and differentiated SH-SY5Y neuroblastoma cells with short or extended exposure to varying concentrations of sAPP $\alpha$ .

RNA was first extracted from the treated cells, cDNA synthesised, and real-time quantitative polymerase chain reaction (rt-qPCR) used to analyse expression. The neuroprotective genes (insulin-like growth factor 2 (*IGF2*) and insulin growth factor binding protein 2 (*IGFBP2*)), and the immediately early genes associated with LTP (*junB*, *zif268* and *BDNF*) were investigated. Expression of *IGFBP2* and *IGF2* in neuroblastoma cells was enhanced by 3- and 4- fold respectively after 30 min exposure to 0.25 nM sAPP $\alpha$ , but with 24 h exposure *IGF2* expression was depressed (0.4-fold) but *IGFBP2* was further enhanced (5-fold). Enhancement of expression for *IGFBP2*

(2.5-fold), *junB* (5-fold) and *zif268* (2.5-fold) was also seen in rat hippocampal cell slices at 1 nM sAPP $\alpha$ .

These results show that sAPP $\alpha$  increases gene expression in genes associated with LTP and neuroprotection. This is consistent with the hypothesis that loss of sAPP $\alpha$  may contribute to the neurodegeneration seen in AD.

**Excitation by GABA in the mouse olfactory bulb is not mediated by a bicarbonate efflux. M Tantirigama, P Heyward. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.**

Gamma-aminobutyric acid (GABA) activates chloride current in neurons, and is the main inhibitory neurotransmitter in the brain. However, recent observations suggest that GABA can depolarise and excite neurons. This could result from regenerative bicarbonate (HCO<sub>3</sub><sup>-</sup>) efflux through chloride channels following strong GABA receptor stimulation. The current study aimed to test this hypothesis *in vitro* in the mouse olfactory bulb.

Transverse mouse olfactory bulb slices (350  $\mu$ m) from 20-30-day-old mice were obtained. Single-cell extracellular recordings were made from the mitral cell layer and the frequency of spontaneous action potential (sAP) firing was recorded. In the first experiment, sAP firing at different concentrations of bath-applied GABA (10  $\mu$ M – 1 mM) was tested. Sixteen of 20 cells (80%) were inhibited at concentrations up to 250  $\mu$ M GABA with four cells displaying no change. At concentrations greater than 400  $\mu$ M, however, 23 of 25 cells (92%) were excited. In many cells, however, this excitation was transient. Some cells displayed rhythmic epileptiform activity across the period of incubation with GABA, while others showed no subsequent firing after an excitatory response. In the second experiment, effect of GABA at 100  $\mu$ M (inhibitory) or 500  $\mu$ M (excitatory) was tested in the presence of acetazolamide (ACTZ), a carbonic anhydrase blocker which disturbs regenerative bicarbonate efflux. Incubation with ACTZ produced no obvious change in epileptiform activity seen at 500  $\mu$ M (n = 8) or inhibition seen at 100  $\mu$ M GABA (n = 5).

In summary, the current study shows GABAergic excitation of mitral cells, similar to that reported elsewhere in the brain. This effect of GABA was not abolished by the blockade of HCO<sub>3</sub><sup>-</sup> efflux, suggesting that at least some GABA synapses on mitral cells may have a depolarising, chloride-mediated, excitatory role in olfactory processing.

**Validation of the electronic nose. Do inhaled salbutamol, exercise, coffee and food affect analysis of exhaled breath? M Tolmay, D Cowan, R Taylor. Department of Medical and Surgical Sciences, University of Otago, Dunedin.**

The electronic nose uses electronic sensors to distinguish odours and analyse exhaled breath. The role of the nose in the diagnosis of asthma is currently being investigated by the Department's Respiratory Research Unit. This study explored the effects of exercise, inhaled salbutamol, and oral intake of coffee and food, on the "smellprint" obtained from the electronic nose to develop guidelines for sampling in clinical studies.

Food intake was a standardised bowl of Sanitarium muesli. Exercise involved cycling for 10 min on an exercise bicycle at 60-80% of maximum heart rate (220-age). Exhaled breath was sampled for 10 sec before and after exposure. The smellprints were then analysed using statistical measures such as the Mahalanobis distance, cross validation value and canonical plots.

Salbutamol, exercise and food intake did not significantly alter the smellprint. However, following caffeine intake, there is a trend towards discrimination of smellprints. This would suggest that prior to breath analysis using the electronic nose, caffeine should be withheld. Further work has demonstrated that sampling time is critical. Samples taken with a 10 sec sampling time differed from those taken over 30 or 60 sec. Thus a sampling time of at least 30 sec and preferably 60 sec is necessary to maximise the possibility of discrimination of different groups by their smellprints. During the main study of the effects of salbutamol, caffeine, food and exercise on the smellprint, a sampling time of 10 sec was used. No significant differences were seen. With longer sampling time, differences might have become apparent and this will be explored in the future.

In conclusion, we recommend that caffeine be withheld prior to breath analysis by the nose and breath be sampled for 60 sec. These changes have been incorporated into the protocol for the use of the electronic nose in our asthma studies.

**Increased cell proliferation in the cochlear nucleus following bilateral cochlear lesions. C Zhang, Y Zheng<sup>1</sup>, P Smith<sup>1</sup>, M Zhang<sup>2</sup>, C Darlington<sup>1</sup>. <sup>1</sup>Department of Pharmacology and Toxicology, and <sup>2</sup>Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.**

Chronic tinnitus is a common condition that significantly reduces quality of life for sufferers. However, few treatments are available, mainly due to a lack of understanding of its mechanisms. Since injury-induced neurogenesis plays an important role in a number of physiological and pathological conditions, we propose that cochlear damage, a common cause for tinnitus, may induce neurogenesis in the cochlear nucleus and that the newborn cells may display different behaviour from the existing neurons. Therefore, the aims of this study were to investigate the time course of cell proliferation in the cochlear nucleus following bilateral cochlear lesions (BCLs) and to identify the phenotypes of the newborn cells.

Thirteen male Wistar rats (n = 3 or 4 per group) were divided into 4 groups: sham surgery without cochlear lesions, BCLs at 24 h, 48 h, and 72 h after surgery. Bromodeoxyuridine (BrdU) was injected at different time points and the animals were killed at 24 h after the injection. Sections (40 µm) were collected throughout the cochlear nucleus using a random systematic sampling method. Immunohistochemistry using anti-BrdU was used to label dividing cells and an optical disector method was used for quantitative analysis.

The number of BrdU<sup>+ve</sup> profiles was found to be significantly increased at 48 h post-surgery (29.9 ± 7.2, mean ± SEM, *P* < 0.01, *t*-test) compared to the sham group (0.8 ± 0.2). Double-immunolabelling revealed that the BrdU<sup>+ve</sup> cells often coexpressed Ki-67, a marker for proliferating cells. However, none of the BrdU<sup>+ve</sup> cells expressed

markers for neuronal stem cells (nestin), immature neurons (doublecortin), or astrocytes (glial fibrillary acidic protein).

Our results provide the first evidence on cochlear lesion-induced cell proliferation and suggest that these proliferating cells may remain at a multipotential status. Such interesting findings may help to develop a target-specific tinnitus treatment in the future.