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Domoic acid-induced cardiotoxicity: a mitochondrial approach. J-H Baek, A Clarkson, A Tramoundanas, B Hesp, IA Sammut, DS Kerr. Department of Pharmacology and Toxicology, OSMS, University of Otago, Dunedin.

Domoic acid (DOM) is a potent excitotoxin, structurally related to the excitatory neurotransmitter, glutamate (Glu). DOM has been shown to cause extensive damage in the central nervous system (CNS), however little is known about its effects within the myocardium.

In vitro, DOM has been shown to affect cardiac mitochondrial respiratory enzymes within the electron transport chain and alter mitochondrial-coupled respiration. Mitochondria have been implicated in excitotoxic damage, and alterations in mitochondria are a fundamental feature of aging. Several studies have shown differences in DOM's neurotoxic effects in young and aged rats. An age-linked decline in the activities of mitochondrial enzymes within brain slices following Glu treatment (1mM) was observed. However, the effect of aging in relation to DOM exposure has not been assessed in cardiac mitochondria.

In this preliminary study, hearts from young (3 months) and aged (27 months) rats, treated with *in vivo* DOM (Young: 0.5mg/kg (n=4), 1.0mg/kg (n=2), 2.0mg/kg (n=2); Aged: 0.5mg/kg (n=2), 1.0mg/kg (n=2), 2.0mg/kg (n=2)) were isolated and freeze clamped. Mitochondrial complex enzyme activities were assessed in the prepared cardiac homogenates to assess mitochondrial impairment. Activities of aged Complex I, II/III, IV, V enzymes and citrate synthase were all decreased by DOM in a concentration-dependent fashion, although there was no significance due to small sample size. Similar results were observed in young, however a decrease was not observed for complex V (ATP synthase) activity.

The aged heart has a lower tolerance than the young heart to oxidative stress, due to its decreased anti-oxidant properties within cardiac mitochondria. Following Glu treatment (1mM), aged rats have been shown to have significantly higher formation of reactive oxygen species (ROS). Mitochondrial complex enzymes have been shown to be targets of free radicals and ROS, and this may explain why decreases in complex enzyme activities were observed in this current study.

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Effect of lactation on prolactin signalling by STAT5b in gonadotrophin releasing hormone (GnRH) and tuberoinfundibular dopaminergic neurons. AS Bang, DR Grattan, GM Anderson. Centre for Neuroendocrinology and Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.

The hormone prolactin plays important roles in mammary gland development and initiating and maintaining lactation, and therefore is found in high levels during late

pregnancy and lactation. This hyperprolactinemic state is maintained by a reduction in the ability of prolactin to activate inhibitory hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons, and by a stimulatory neuroendocrine reflex evoked by suckling of the young. One of the many other effects of hyperprolactinemia is infertility; however how prolactin acts in the brain to achieve this is not understood. Activation of prolactin receptors in TIDA neurons can be detected by staining for its cytoplasmic transcription factor, STAT5b. The aims of this project were to determine: (1) if prolactin signals via STAT5b in GnRH neurons, which govern reproduction, and (2) whether lactation alters the sensitivity of GnRH and TIDA neurons to prolactin.

Three groups of female rats (n = 9-10 per group) were used: diestrous (normally cycling), lactating with pups removed for 4 h to acutely reduce endogenous prolactin levels, and lactating with pups removed for 24 h. These were each divided into two subgroups, one receiving a single prolactin injection (250 µg) and the other vehicle 45 min before brain collection. Sections containing the arcuate nucleus and preoptic areas were stained by immunohistochemistry to identify prolactin-induced translocation of STAT5b into the nucleus of TIDA or GnRH neurons, respectively.

In diestrous rats, treatment with prolactin induced nuclear STAT5b translocation in TIDA neurons. However these neurons were insensitive to prolactin during lactation. Prolactin did not induce STAT5b signalling in GnRH neurons in either diestrous or lactating rats. These results demonstrate that (1) prolactin does not act directly on GnRH neurons, at least via the STAT5b signalling pathway, and (2) during lactation prolactin signalling is suppressed in TIDA neurons so that hyperprolactinaemia can be maintained.

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A new approach to the delivery of RNA interference to human cells. CY Chan, D Markie. Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin.

RNA interference (RNAi) is the specific inhibition of gene expression by double-stranded RNAs. One established approach is the *in vivo* expression of short hairpin RNA (shRNA) molecules from plasmid vectors in mammalian cells to induce loss-of-function in various biological systems. The present study describes the development of a novel human RNAi system using the Polymerase Chain Reaction (PCR) that can be controlled as required, making it more efficient than previous RNAi methods.

A template suitable for generating DNA fragments containing both a selectable marker and the shRNA required to knockdown specific genes was constructed. By PCR, we obtained DNA fragments with shRNA targeted against the human *BUB3* gene from this template. We then transfected these fragments into TREX-293 human cells, where they were successfully integrated into the cell chromosomes to create stable cell lines. This result demonstrates that PCR may be useful in generating fragments for this purpose.

In our attempt to knockdown the human *BUB3* gene, the TREX-293 cells still exhibited normal levels of *BUB3* protein, indicating that knockdown was initially

unsuccessful. This may be due to either a failure in the chosen RNA sequence to function effectively as an RNAi template, or a failure of shRNA expression from the fragment, and further experiments using alternative shRNA sequences will be required to answer this question.

However, despite the lack of success with BUB3, we did develop a method that allows us to produce DNA fragments for RNAi by PCR with the potential for carrying out rapid functional analyses of genes. This, therefore, is a first step in the development of assays which can then be used to evaluate novel genes with unknown functions.

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Defining neuropeptide Y interactions with gonadotrophin-releasing hormone neurons in mice. E Cottrell, R Campbell, A Herbison. Department of Physiology, Centre for Neuroendocrinology, Otago School of Medical Sciences, University of Otago, Dunedin.

Reproductive function is governed by a population of cells within the brain, the gonadotrophin-releasing hormone (GnRH) neurons. These cells receive a multitude of signals reflective of the physiological state of the individual. The study of the regulation of networks governing these neurons is of importance in understanding the regulation of fertility, and how this is restricted to appropriate circumstances.

Neuropeptide Y (NPY) is one molecule identified as playing a role in regulation of GnRH neuron activity, and proposed as a potential signal in the integration of energy balance and reproductive function. We have been investigating the potential role of the NPY Y1 receptor (Y1R) subtype in the regulation of mouse GnRH neurons, as recent studies have found that the Y1R colocalised with GnRH nerve terminals in the rat.

Mice used in these studies were anaesthetised with sodium pentobarbital and killed by transcardial perfusion with 4% paraformaldehyde fixative solution. Brains were then rapidly dissected out, post-fixed and processed for immunohistochemical (IHC) study. Antibodies used were rabbit anti-NPY Y1R (directed against either the C- or N-terminal regions), sheep anti-GnRH and rabbit anti-galanin. Single-label IHC with Y1R antibodies was done firstly in male animals, to optimise antibody conditions and define Y1R distribution. Following this, double-label IHC with confocal microscopic imaging was employed in female mice to investigate Y1R expression on GnRH neurons. Galanin, a peptide molecule expressed in GnRH cells, was used as a positive control for colocalisation. Of a total of 208 GnRH neurons from five female animals analysed using confocal microscopy for GnRH/Y1R colocalisation, none were found to show convincing Y1R expression. This absence of coexpression was substantiated using a GnRH/galanin control.

Given this negative result, we propose a scenario where NPY may exert indirect effects on the GnRH neuronal system to regulate reproductive function.

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Localisation of prolactin receptor mRNA in identified magnocellular neurons in the female rat brain using dual-label *in situ* hybridisation histochemistry.

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We have previously identified the receptors that mediate the action of the hormone prolactin in several areas of the hypothalamus including the supraoptic and paraventricular nuclei. The aim of this study was to identify the neurochemical phenotype of the magnocellular neurons that express prolactin receptor mRNA in these two nuclei. Furthermore, as the supraoptic and paraventricular nuclei both undergo significant plasticity during lactation, we examined whether prolactin receptor expression changed during this time.

Dual-label *in situ* hybridisation histochemistry was performed on 3-4 sections per brain structure from lactating (n=3-5) and non-pregnant (n=3-5) female rats. Sections were hybridised with a ³⁵S labelled nucleic acid probe that specifically detected the long form of the prolactin receptor together with non-radioactive (digoxigenin-labelled) RNA probes to detect either oxytocin or vasopressin mRNA. Following visualisation of digoxigenin-labelled probes by immunohistochemistry, sections were coated with photographic emulsion and stored at 4°C for 4 weeks before being developed to detect prolactin receptor mRNA. Images were analysed using NIH image software.

In non-pregnant rats, 87 ± 5% (mean ± S.E.M.) of the oxytocin magnocellular neurons in the supraoptic nucleus and 51 ± 8% of neurons in the paraventricular nucleus expressed prolactin receptors. The proportions of neurons showing co-localisation did not change significantly in the lactating group. In contrast, prolactin receptor mRNA was present in less than 25% of vasopressin neurons in both hypothalamic nuclei of non-pregnant animals (24 ± 10% in the paraventricular, and 14 ± 4% in the supraoptic, nucleus) and lactating animals (16 ± 2% in the paraventricular, and 20 ± 5% in the supraoptic, nucleus). The detection of prolactin receptors on oxytocin and to a lesser extent on vasopressin neurons implies prolactin can directly modulate the activity of magnocellular neurons and supports data that suggests prolactin may have important brain actions in addition to its role in the establishment and maintenance of lactation.

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The effects of anti-psychotic drug-induced hyperprolactinaemia on reproductive neuroendocrine function in female rats. DC Kieser, DR Grattan, GM Anderson. Centre for Neuroendocrinology and Department of Anatomy and Structural Biology, Otago School of Medical Sciences, University of Otago, Dunedin.

It is known that hyperprolactinaemia, a common side-effect of many antipsychotic drugs, causes infertility and loss of libido in humans and animals. The underlying mechanisms of this effect are largely unknown. Insight into these mechanisms could lead to better therapies for pathological and antipsychotic drug-induced infertility,

production of improved antipsychotics that avoid this side-effect, or conversely, the generation of new non-steroidal methods for suppressing fertility in both males and females. We investigated whether chronic anti-psychotic drug-induced hyperprolactinaemia inhibited three neuroendocrine parameters necessary for female fertility: the surge of gonadotrophin releasing hormone (GnRH) and luteinizing hormone (LH) that induces ovulation, tonic pulsatile secretion of LH, and the negative feedback of oestradiol on LH pulses.

Ovariectomised rats (n = 5-6) received sulpiride (1.25 mg sc) or vehicle twice-daily for 8-10 days, resulting in marked hyperprolactinaemia. When also treated with oestradiol to mimic the presence of ovarian oestrogens, the frequency of LH pulses was suppressed in hyperprolactinaemic rats ($p < 0.05$). This did not occur in the absence of oestradiol. There was no effect of sulpiride on LH pulse amplitude under either steroidal condition. When rats were acutely treated with doses of oestradiol and progesterone known to induce a preovulatory-like GnRH/LH surge, the peak plasma concentration of LH and the activation of GnRH neurons (as determined by immunocytochemical detection of the neural activity marker Fos in GnRH neurons) were not significantly different between sulpiride- and vehicle-treated rats.

We conclude that the inhibitory effect of hyperprolactinaemia on LH pulse frequency requires the presence of ovarian steroids, and that hyperprolactinaemia does not markedly inhibit the preovulatory surge of GnRH and LH.

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Herbal products: a follow-up survey of opinions, perceptions and behaviours of callers to the New Zealand National Poisons Centre. J Lee, N Smith. Department of Pharmacology & Toxicology, Otago School of Medical Sciences, University of Otago, Dunedin.

Health professionals have expressed concerns about consumer misconceptions that herbal products (HP) are “natural, safe and non-toxic”. This study investigated the attitudes, opinions and behaviours towards HPs via a follow-up telephone survey of the 98 general public callers who contacted the National Poison Centre (NPC) regarding HPs, between July 2002 and November 2003. The 60 respondents were 95% female, 73% aged 21-40 years, 32% university educated and 93% of New Zealand European descent.

Two-thirds of respondents recalled no additional advice provided when purchasing HPs, consistent with almost half obtaining HP from sources where professional advice was not available (e.g. supermarket). HPs were used primarily in disease prevention (66.7%). Most respondents (58.3%) did not believe that HPs were more efficacious than conventional medicine (CM), but favoured HPs for their perceived safety. Just over half (55%) also believed that combining HPs with CM result in increased efficacy, compared to when using either independently. Only 15% of all reported products had child safety packaging. Only 43% of HPs were stored with CM but few (13%) of HPs were actually locked away.

Most respondents were willing to tell health professionals about their HP use and adverse reactions. NPC advice was considered by respondents as very useful (70%),

sufficient in quantity (63%) and very clear (62%). Almost all (98%) were satisfied with the NPC service and would recommend it to others.

The study findings emphasise the need for health professionals to discuss safe use and storage of HPs with patients, and caution them against over-estimating HP safety, to minimise problems associated with their use. Child safety packaging for HPs also needs greater promotion. It is anticipated that as HP use becomes more popular there may be a corresponding increase in HP poisonings, and the NPC database should acquire more detailed information to meet this need.

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Neuroprotective effect of (-)-epigallocatechin gallate in a rat model of hypoxia-ischaemia-induced brain damage. BA Sutherland, O Shaw, AN Clarkson, I Appleton. Department of Pharmacology & Toxicology, Otago School of Medical Sciences, University of Otago, Dunedin.

(-)-Epigallocatechin gallate (EGCG) is a polyphenolic antioxidant that protects cells against free radical damage. It was previously shown that 50 mg/kg EGCG is neuroprotective in a rat model of hypoxia-ischaemia (HI). This study investigated the possible mechanisms underlying the neuroprotective effects of EGCG.

The left common carotid artery was permanently double ligated in 26 day old male Wistar pups (n = 8). Two hours later, the rat was placed in an 8% O₂/92% N₂ atmosphere for 60 minutes. This produced an infarction on the ipsilateral side of the brain. There were three treatment groups: untreated, with no HI (control); HI + 0.9% saline; and HI + 50 mg/kg EGCG. Treatments were administered i.p. daily beginning one day prior to HI for 4 days. 29 day old rats were euthanised in accordance to ethical guidelines.

Western blot analysis found that HI did not alter the protein levels of neuronal nitric oxide synthase (nNOS) or endothelial NOS (eNOS) significantly compared to controls. Inducible NOS (iNOS) was significantly increased after HI (0.22 ± 0.06 optical density (OD)) compared to controls (0.06 ± 0.02 OD; $P < 0.05$; unpaired *t*-test) but decreased again with EGCG administration (0.12 ± 0.03 OD). HI + EGCG significantly increased nNOS (0.42 ± 0.06 OD) and eNOS levels (2.18 ± 0.6 OD) compared to HI + saline (nNOS: 0.27 ± 0.05 OD, $P < 0.05$, unpaired *t*-test; eNOS: 0.68 ± 0.27 OD, $P < 0.05$, unpaired *t*-test).

Previous experiments have identified that nitric oxide (NO) derived from eNOS is neuroprotective, whereas NO from iNOS and nNOS is neurotoxic. Therefore, the neuroprotective effects of EGCG may partly be due to increased eNOS levels and decreased iNOS levels suggesting that EGCG produced its neuroprotection by modulating NOS isoforms. This further substantiates that EGCG is an effective neuroprotectant in neurodegenerative disorders such as HI.

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Molecular characterisation of anginolysin A, a lytic bacteriocin produced by *Streptococcus anginosus*. Y-T Ting, M Dufour, N Heng, J Tagg. Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, Dunedin.

The oral bacterium *Streptococcus anginosus* produces anginolysin A, a cell wall-degrading (lytic) antibacterial protein (bacteriocin) that kills *Streptococcus pyogenes*, the causative agent of streptococcal sore throats. The objectives of this research project were: (i) to use molecular biological techniques to characterise the genetic locus for anginolysin production from two *S. anginosus* strains T-29 and H19, (ii) to compare and contrast the deduced amino acid sequence of anginolysin A with that of zoocin A, the prototype streptococcal lytic bacteriocin, and (iii) to clone the anginolysin gene into an expression vector for protein overexpression in *Escherichia coli* hosts.

Using PCR primers targeting a highly-conserved region within the catalytic domain of lytic enzymes, a PCR product (designated Pep) was obtained. The nucleotide sequence of the Pep product was subsequently used in the design of new inverse PCR primers in order to obtain the rest of the anginolysin A gene (*angA*) as well as the associated bacteriocin immunity gene (*angI*). The function of the *angA* gene was confirmed as gene knockout mutants no longer produced anti-*S. pyogenes* bacteriocin activity.

The anginolysin and zoocin protein sequences are very similar (73%) with the main amino acid differences observed in their substrate-binding (target recognition) domains, which may explain why anginolysin A kills a much narrower range of target bacterial strains. Furthermore, the gene arrangement of the anginolysin A locus was different to that of zoocin A.

Finally, the *angA* genes of strains T-29 and H19 have been cloned into the expression vector pQE-80L to facilitate protein overexpression experiments in *E. coli*. This will allow further biochemical characterisation of purified anginolysin A proteins.

In conclusion, this study has identified a new member of the lytic class of bacteriocins. The information obtained provides the foundation by which future experiments aimed at elucidating structure-function relationships of lytic bacteriocins can be designed.

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Inflammatory cytokine profile during total hip arthroplasty: a pilot study. J Yap, JC Theis. Department of Orthopaedic Surgery, Otago School of Medicine, University of Otago, Dunedin.

Cytokines are molecules in our bodies which are involved in our immune system. Total hip arthroplasty, otherwise known as hip replacement surgery is a common cause of fat embolism, which occurs when pressure in bone cavities displaces fat and bone marrow into the blood circulation. Because of the possible involvement of cytokines in fat embolism, this pilot study was designed to investigate the cytokine profile of subjects who went through total hip replacement surgery.

Blood samples were obtained from a peripheral vein in five subjects at 8 different times during and after a total hip replacement surgery within a period of 72 hours. Three subjects had a routine cemented surgical procedure, one had a partial cemented procedure and one went through an uncemented procedure. Serum levels of three inflammatory cytokines, Interleukin (IL)6, IL10 and IL1 β , were measured using an ELISA. Haemodynamic parameters, i.e. blood pressure, oxygen saturation and pulse were measured at similar times when the blood was taken.

The study showed a marked increase in inflammatory cytokines. Very low levels of inflammatory cytokines were detected before and during the operation in all five of the participants. Post-operatively, there was a continuous rise in IL6 concentration, peaking at 6-12 hours followed by a steady decline towards baseline values. There also was a steady and continuous rise in concentration of the anti-inflammatory cytokine IL10, peaking at 12-48 hours after surgery. In the present study, IL1 β levels did not change appreciably. The study found no significant correlation between any of haemodynamic parameters and their corresponding cytokine response.

From this pilot study, it can be concluded that total hip replacement surgery causes an increase in inflammatory cytokines. Future studies could evaluate whether a cemented procedure results in a significantly greater inflammatory response.

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