Escherichia coli physiology is its flexible respiratory metabolism which stems from an elaborate bank of membrane-associated respiratory enzymes. Intriguingly, many respiratory enzymes are located outside of the cell cytoplasm and are often extremely complex consisting of multiple subunits and associated redox cofactors. How are such enzymes assembled and exported?

Many eubacteria and archaea (and their chloroplast descendants) have the ability to transport pre-folded, very often oligomeric, and enzymatically active proteins across ionically-sealed membranes. The proteins so-transported are usually synthesised with distinctive N-terminal signal peptides that bear a common 'twin-arginine' SRRxFLK amino acid sequence motif. All proteins bearing twin-arginine signal peptides are transported by the **twin-arginine translocation (Tat) system** (1). The Tat translocase is essentially a membrane-bound nanomachine dedicated to the translocation of fully folded proteins and, depending on the biological model system under investigation, comprises 2 or 3 different membrane proteins. In *E. coli*, 3 types of integral membrane proteins – TatA (and its homolog TatE), TatB, TatC - are involved in the transport process (1). Two distinct Tat complexes can be isolated from resting *E. coli* inner membranes. TatA can be purified as a large, heterogeneous complex that also contains trace amounts of TatB (2), and the TatC protein is found in another large complex with an equimolar amount of TatB. The TatBC complex is the 'signal recognition module' that contains the recognition site for the twin-arginine motif on the signal peptide. Low-resolution structural analysis points to the TatA protein forming the protein-conducting channel (or 'transport module') of the Tat system. During protein transport a transient TatABC complex is thought to form in the membrane, but then rapidly dissociate again once the translocation of the substrate has been completed. The whole system is probably powered directly by the transmembrane proton motive force.

The central dogma of Tat transport is therefore that Tat substrates are fully folded before export. As a result it is essential that biosynthesis and assembly of complex respiratory enzymes is completed in the cytoplasm before the final transport event is even attempted. A subset of Tat-targeted proteins have been found to be integral membrane proteins suggeting that the Tat translocase also has the ability to recognise and integrate transmembrane segements into the lipid bilayer (3). In addition, recent work has unearthed a mechanism that prevents premature targeting of Tat signal-bearing proteins until all biosynthetic processes are concluded. This 'Tat proofreading' process involves the pre-export interaction of Tat signal peptides with dedicated binding proteins (4,5). One such Tat proofreading chaperone is TorD, a small multi-functional protein, that binds tightly to the Tat signal peptide of trimethylamine *N*-oxide reductase (TorA) (4,5). TorD-like chaperones have a unique all-helical fold and are almost ubiquitous in bacteria and archaea. Recently, however, it has emerged that second family of peptide binding proteins, completely unrelated to TorD in terms of structure, also operates in bacteria in a Tat proofreading capacity. The NapD chaperone has a ferredoxin-like fold and binds specifically to the Tat signal peptide of nitrate reductase (NapA) preventing export until all assembly processes are complete.

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I dedicate the 2007 Colworth Medal Lecture to my wife Tracy Palmer and our boys James and Jack. I would like to thank Tracy in her capacity as Professor of Molecular Microbiology at the John Innes Centre, Norwich, and Ben C Berks, University of Oxford, for expert guidance through the postdoc years and excellent collaborations thereafter. I accept this tremendous award on behalf of each of us and all of our hard-working staff both past and present. I also thank the University of East Anglia, and in particular David J Richardson, for supporting and mentoring me. I acknowledge The Royal Society, the BBSRC, and the John and Pamela Salter Charitable Trust for financial support.

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C73 & PC137

Endothelial galectin-1 is a novel determinant of endogenous anti-inflammation

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Galectin-1 (Gal-1) is a β-galactoside binding protein, the expression of which is increased in endothelial cells upon exposure to pro-inflammatory stimuli (Baum LG *et al.* 1995). Through binding of several receptors (CD7, CD45 and CD43) Gal-1 is known to induce apoptosis of activated T lymphocytes, an effect thought to mediate the beneficial effects of Gal-1 in various inflammatory models. The data presented here highlights another function for Gal-1; that of a negative regulator of T cell recruitment to the endothelium, both under physiological and pathophysiological conditions. We have shown, using siRNA to knockdown Gal-1 in endothelial cells, that endogenous Gal-1 limits T cell capture, rolling and adhesion to activated endothelial cells under flow. Indirect binding and inhibitor studies suggest that Gal-1 acts on CD45 and consequently causes inhibition of the src kinase p56Lck to bring about its inhibitory effect on lymphocyte adhesion. These findings are corroborated by studies in Gal-1 null mice in which homing of wild type T lymphocytes is significantly increased to mesenteric lymph nodes and to the inflamed paw in a model of delayed type hypersensitivity. In conclusion, mimicking endothelial Gal-1 actions would be a novel strategy for controlling aberrant T cell trafficking, hence for the development of anti-inflammatory therapeutics.

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C74 & PC168

Calsenilin regulates Amyloid-β formation and transient 'A'-type current through Kv4.2 subunits

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Calsenilin is a presenilin-binding protein, increasing γ-cleavage of the Alzheimer amyloid protein precursor, thereby elevating the amyloid β protein (Aβ)[1]. Calsenilin is also a Kv4 channel interacting protein (KChIP3), responsible for protein trafficking of Kv4 channels[2]. We have previously shown that Aβ increases expression of 'A'-type K+ channel currents in rat cerebellar granule neurons[3]. This increase is due to the upregulation of the Kv4.2 subunit in particular[4]. Here we investigate the effect of soluble Aβ1-40 on the Kv4.2 subunit and the importance of its association with KChIP3. Dissociated cultures of cerebellar granule neurones (CGN) were prepared from 6-8 day old rats. Whole-cell patch clamp measurements of K+ channel currents were carried out using quasi-physiological intra- and extracellular solutions[3]. Stock Aβ was solubilised in DMSO before dilution in culture media to a concentration of 10nM and applied to cultures for 24 hours. Immunopharmacological techniques were employed by adding antibodies into the intracellular pipette solution. This would preferentially block the protein of interest enabling functional properties to be investigated. Protein expression for KChIP3 and Kv4.2 in the presence of Aβ (10nM, 24hours) were assayed using western blotting. The importance of endogenous Aβ was considered by application of the γ-secretase inhibitor, γ-I (10μM, 24 hours) which blocks the production of Aβ1-40 at the presenilin complex. Statistical differences were assessed using repeated measures ANOVA with Tukey's posthoc test or unpaired Student's t-test as appropriate.

Soluble rat recombinant Aβ1-40 caused a significant increase in peak K+ current density/voltage (I-V) relationships, similar to previously reported data[5]. This increase was significantly blocked by 35.38±0.9 % in the presence of the intracellular Kv4.2 antibody (n=10 control Kv1.4 antibody, 10 Kv4.2 antibody $p<0.001$). At a test potential of 50mV, current decreased from 0.86±0.07 nA/pF to 0.56±0.05 nA/pF. In another set of similar experiments the increase in peak K+ current of Aβ1-40 treated cells were significantly blocked by 19.5±0.93%, in the presence of the intracellular KChIP3 antibody (n=11 control KChIP2 antibody, 10 KChIP3 antibody, $p<0.05$). Western blotting revealed a 30.0±0.1% increase in KChIP3 protein levels after Aβ1-40 treatment (n=4 control, Aβ treated, $p<0.05$). When endogenous Aβ1-40 levels were inhibited by γ-I, protein expression of KChIP3 significantly increased by 56.0±0.1% (n=3 control, γ-I treated cells, $p<0.05$). These data suggest the increase in 'A'-type K+ current in the presence of Aβ1-40 is due to involvement of the Kv4.2 subunits by augmenting its association with KChIP3.

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C75 & PC276

Subcellular distribution of key proteins involved in endothelin-1-induced arrhythmia and inotropy in adult rat ventricular myocytes (ARVMs)

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Calcium (Ca^{2+}) is a highly versatile signal that can regulate many different cellular functions. At any moment in time, the intracellular concentration of Ca^{2+} is tightly regulated through a series

of 'on' and 'off' reactions, which either release Ca^{2+} from the sarcoplasmic reticulum (SR) and/or endoplasmic reticulum (ER) into the cytoplasm or remove this signal by a combined action of pumps and exchangers. This cyclical movement of Ca^{2+} underlies the regular contraction of heart myocytes. Modulation of Ca^{2+} circulation (e.g. by the vasoactive peptide endothelin-1 or the β-adrenergic agonist isoproterenol) alters cardiac muscle contractility resulting in inotropic, lusitropic, or chronotropic effects. However, the effects of such stimuli can also initiate disadvantageous responses. For instance, Ca^{2+} dysregulation during disease in humans and other animals can lead to abnormal contraction of the cells and cardiac arrhythmias.

We have recently shown that the intracellular distribution of certain of the proteins and organelles involved in Ca^{2+} signalling determines the nature of agonist-induced Ca^{2+} signals in adult rat cardiac myocytes (Mackenzie et al., 2004). We have now extended these studies to determine the relative subcellular distribution of key proteins involved in endothelin-1-stimulated $InsP_3$-dependent Ca^{2+} release and Ca^{2+}-induced Ca^{2+} release in cardiac myocytes. To this end, we have used confocal microscopy to visualise the intracellular distribution of the endothelin A and B receptors (ET_AR and ET_BR), the inositol 1,4,5-triphosphate receptor ($InsP_3R$), ryanodine receptor (RyR), L-type channel (LTC) and Na/Ca Exchanger (NCX). These studies showed that $InsP_3Rs$ are distributed primarily around the nuclei and under the plasma membrane. This distribution is unlike that observed for the RyR, which was found throughout the cell. LTCs and NCX were distributed on the plasma membrane. ET_ARs were located on the plasma membrane throughout the cell, whereas ET_BRs were found only on the plasma membrane at the polar ends of the cell. In addition, to gain insight into how $InsP_3Rs$ and RyRs are differentially distributed on intracellular Ca^{+2} signalling organelles, we have performed confocal immunofluorescence studies with antibodies raised against proteins found exclusively on the ER. These studies indicated that the ER and the SR in cardiac myocytes are distinct organelles, adding a further level of complexity to the understanding of Ca^{+2} signalling in these cells.

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C76 & PC338

Electrophysiological characteristics of freshly isolated ventricular myocytes from Zebrafish *(Dario rerio)*

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The Zebrafish is a tropical teleost fish that has been the focus of increasing numbers of developmental studies. Physiological interest in this species has been spurred by the ease of mutants can be induced by chemomutagenesis. However, our understanding of the basic physiology of Zebrafish, in particular cardiac excitation-contraction coupling is limited. Indeed, there is currently no information about the electrical activity of single myocyte freshly isolated from Zebrafish ventricle heart; this study addressed this point. Heart was removed post mortem from Zebrafish and ventricle was then minced and stir gently in isolating solution containing trypsin and collagenase for 30 min. The extracellular physiological solution was (in mM): 150 NaCl, 5.4 KCl, 1.5 MgSO4, 0.4 NaH2PO4, 2 CaCl2, 10 glucose, 10 HEPES (pH 7.7 with NaOH). The whole cell configuration of the patch clamp was used to record Na current (INa), Ca current (ICa) and action potential (AP). Patch pipettes were filled with internal solution (in mM): 139 KCl, 10 NaCl, 1 MgCl2, 20 HEPES, 5 MgATP, 0.3 NaGTP (pH 7.2 with KOH). Cell capacitance was determined using the membrane test protocol of Clampex. During voltage clamp experiments, holding potential was -80 mV and stimulation frequency was 0.1 or 1 Hz. Results are presented as mean±SE and analyzed with paired t-test. Single ventricular myocytes from Zebrafish are long and thin, as described for other fish species. Cell capacitance was 27.4±2.5 pF (n=17). ICa density (test pulse to 0 mV) was -11.2±2.9 pA/pF and the time to reach 37% of ICa peak was 29.2±2.6 ms (n=7). INa density (test pulse to -40 mV) was -104±22 pA/pF (n=5). Increasing stimulation frequency to 1 Hz decreased peak INa by 10±5% and increased peak ICa by 20±7% ($p<0.05$). At 0.1 Hz, the resting membrane potential and AP overshoot were -79.3±0.3 mV and 20.8±4.9 mV, respectively (n=7). dV/dt was 42±11 V/s and AP duration at 25, 50, and 90% repolarisation was 21±6, 32±8, 39±9 ms, respectively (n=7). Increasing stimulation frequency to 1 Hz does not significantly alter these parameters ($p>0.05$). However, increasing stimulation frequency to 2 Hz significantly decreased AP overshoot ($n=6, p<0.05$) and reduced dV/dt ($n=6, p=0.06$). To conclude, we have developed a method to obtain viable isolated ventricle myocytes from Zebrafish heart. Ionic currents studied present characteristics similar to other fish species. The presence of a plateau during the AP suggests that this species might be appropriate for ion channels related mutation screening of cardiac alteration.

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C77 & PC393

Hypothalamic glucokinase plays a critical role in the regulation of protective feeding to glucoprivation

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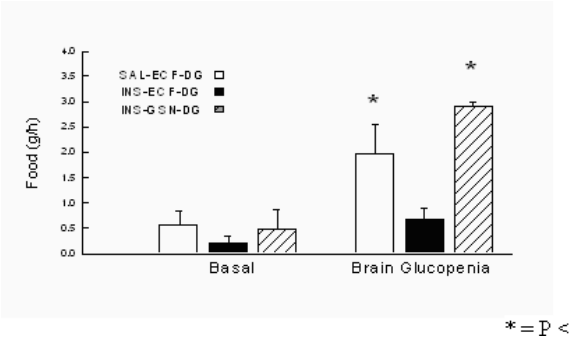
Hypoglycaemia or glucopenia trigger protective responses including the stimulation of feeding. These responses may become impaired in some type 1 diabetic patients following aggressive insulin therapy. We hypothesized that glucoprivation

might be sensed by hypothalamic glucose-sensing neurones using the specialised glucose-sensing molecule glucokinase (GK) and have recently shown that ICV delivery of GK inhibitors such as glucosamine (GSN) boosts feeding in Sprague Dawley (SD) rats (Evans *et al.*, 2005). Here, we examined whether low dose GSN (15 nmol/min- below the orexigenic dose) can boost defensive feeding when delivered in combination with 2-deoxyglucose (2DG) induced glucopenia.

We created a model of hypoglycaemia unawareness in male SD rats by exposure to 3 days of insulin injections [INS] or saline [SAL] in controls. We measured feeding responses on day 4 to brain glucopenia (ICV 2DG) with/ without GSN. We studied three groups of animals: (1) SAL injected rats with day 4 ICV vehicle and 2DG (SAL-ECF-2DG) (2) INS injected rats with day 4 ICV vehicle and 2DG (INS-ECF-2DG) or (3) INS injected rats with day 4 ICV GSN and 2DG (INS-GSN-2DG). Antecedent INS injections suppressed glucoprivic feeding response (INS-ECF-2DG vs SAL-ECF-2DG- see figure). ICV GSN restored feeding response to a level similar to that seen in control rats (see figure). We then reasoned that glucoprivic feeding would be impaired in mice deficient in GK. GENA348 mice with defective GK (Toye *et al.*, 2004) displayed markedly blunted incremental feeding response to glucopenia following intraperitoneal 2DG injections relative to wild type BALB-C mice (table). Together, our data suggest a role for hypothalamic GK in the control of protective feeding responses to glucopenia. These findings also imply that hypothalamic GK may perhaps represent a novel target for restoring protective hunger symptoms to hypoglycaemia in type 1 diabetes.

Glucoprivic feeding response in GENA348 (GK mutant) and BALB-C (Wild-type) mice.

	BALB-C (n = 5 to 9) (Mean ± SEM)	GENA348 (n = 3 to 9) (Mean ± SEM)
Food intake 3hours after saline injection (g)	0.22 ± 0.02	0.1 ± 0.06
Food intake 3hours after 2-DG injection (g) P = 0.06	0.4 ± 0.11	0.16 ± 0.04
Incremental feeding response to glucopenia (g) P = 0.09	0.24 ± 0.08	0.08 ± 0.04



* = P < 0.05

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C78 & PC540

Characterisation of the pro-aggregatory effects of gram positive or gram negative bacteria in human platelet rich plasma

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Platelets are non-nuclear cell fragments that are involved in innate immunity and inflammation. Platelets express pattern recognition receptors including Toll-like receptors (TLR) 2, 4 and 9 (Cognasse et al., 2005) that are utilised by tissues to sense Gram positive and Gram negative bacteria. However, reports regarding the effects of TLR activation or bacteria on platelet activation are conflicting (Ward et al., 2005; Matera et al., 1992). In the current study we have investigated the effects of Gram positive bacteria, *S.aureus*, or Gram negative bacteria, *E.coli*, on the aggregation of human platelets. We have gone on to characterise the nature of the aggregatory response induced by bacteria using aspirin, the stable prostaglandin I_2 mimetic treprostinil sodium, and the nitric oxide donor SIN-1, the three of which inhibit platelet activation by different mechanisms.

Platelet rich plasma (PRP) was prepared and 100µl added to the individual wells of 96-well plates. Aggregation was determined by measuring the changes in absorbance of individual wells at 595nm for 16 minutes at 37°C.

S.aureus and *E.coli* (10^9cfu/ml) stimulated aggregation by 61±1% and 62±7%, respectively (n=3). Addition of 10^{-5}M ADP increased the aggregations to *S.aureus* and *E.coli* by 24% and 29%, respectively (n=3). Similar increases were seen following the addition of arachidonic acid ($3x10^{-5}M – 3x10^{-3}M$) or the thromboxane A_2 mimetic, U46619 ($10^{-7}M – 10^{-5}M$) (n=3). Treprostinil sodium inhibited the aggregatory responses to the bacteria in a concentration-dependent manner, with log IC_{50} values of -8.0±1.6 and -8.8±0.5 against *S.aureus* and *E.coli*, respectively. In contrast, bacterial-induced aggregation was not inhibited by aspirin (n=3). As for treprostinil sodium, SIN-1 inhibited bacterial-induced platelet aggregation, with log IC_{50} values of -5.9±0.1 and -6.3±0.2 against *S.aureus* and *E.coli* respectively.

Our findings that *S.aureus* and *E.coli* stimulate platelet aggregation and that these aggregations are inhibited by SIN-1 or treprostinil sodium, but not aspirin, may have important implications in the treatment of bacterial diseases in which platelets play key roles such as sepsis.

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