Poster Session 3 – Pharmacology

211
Toxicity of glucose analogue 2-deoxy-D-glucose mediated through hypoxia-induced glucose transporter Glut-1 in human cervix carcinoma cells

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Hypoxia is a major therapeutic problem, causing chemo- and radio-resistance, increased malignancy and metastasis. The ability of tumour cells to flourish in this adverse microenvironment is chiefly mediated by a switch to anaerobic glycolysis (Airley *et al* 2000), via hypoxia-inducible facilitative glucose transporter Glut-1. Glut-1 is overexpressed and correlates with poor prognosis in virtually all tumours (e.g. in cancers of the head and neck and cervix) (Airley *et al* 2001; Oliver *et al* 2003). Hence, it is an interesting target for novel diagnostic and therapeutic anticancer strategies.

Overexpression of Glut-1 may be exploited by the use of glycolytic inhibitors (e.g. glucose analogue 2-deoxy-D-glucose (2-DG)), or via direct binding and inhibition of Glut-1, thereby inhibiting the flow of glucose into the cell. Isoflavone tyrosine kinase inhibitors (e.g. quercetin) (Vera *et al* 2001) exert this effect. Previously, we observed that the CaSki cervix carcinoma cell line shows low constitutive levels of Glut-1, but that upon exposure to hypoxia (1% O_2), the level of Glut-1 is significantly increased. In this preliminary study, the role of Glut-1 in the uptake and toxicity of 2-DG (dose range 0–500 um), determined by MTT proliferation assay, was investigated using CaSki cells primed in normoxic and hypoxic conditions. To test the additional hypothesis that a Glut-1 binding agent might inhibit the entry of 2-DG into the tumour cells, and therefore its toxic effects, cells were exposed to 2-DG in the presence or absence of 20 um quercetin.

Preliminary data shows trends where exposure to hypoxia for 18 h before treatment with 2-DG increased toxicity, causing an approximate 2-fold decrease in IC50. The cytotoxic effects of a 2-DG and quercetin combination, however, seemed to depend upon the conditions used, and therefore the amount of Glut-1 present, and the dose of 2-DG. In hypoxia, addition of quercetin seemed to attenuate the effect of 2-DG, possibly due to inhibition of 2-DG uptake. However, the most significant effect occurred where addition of quercetin to cells primed in normoxia increased 2-DG toxicity ($P = 0.05$), the greatest effect observed at the lower end of the dose range of 2-DG, indicating possible additive or synergistic effects where 2-DG uptake would be limited by the low level of Glut-1 present.

In conclusion, hypoxia may increase toxicity of the glucose analogue 2-DG via upregulation of Glut-1. The therapeutic merit of a 2-DG/Glut-1-binding agent combination, however, may depend upon the level of oxygenation. This preliminary data supports the contention that tumour oxygenation may impact on response to Glut-1 or glucose-dependent drugs, and that targeting Glut-1 may be a means of achieving selective toxicity to hypoxic tumours.

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212
Relationship between markers of hypoxia, MYCN amplification and survival in paediatric neuroblastoma and rhabdomyosarcoma

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Tumour hypoxia leads to poor prognosis, possibly as a result of induction of hypoxia-regulated genes which enable adaptation to these adverse conditions. For example, the facilitative glucose transporter Glut-1 predicts poor prognosis in many tumours, whereas the angiogenic growth factor Vascular Endothelial Growth Factor (VEGF) stimulates the production of new blood vessels (Airley *et al* 2000). Tumour hypoxia is traditionally a problem in adult solid tumours, and we have established Glut-1 as a useful surrogate marker of hypoxia in cervix carcinoma (Airley *et al* 2001, 2003). However, there is little information regarding the influence of hypoxia in paediatric cancers. To address this, expression of Glut-1, VEGF, their relationship with prognostic indicators such as the oncogene MYCN, and overall survival, are being investigated in patients with paediatric neuroblastoma and rhabdomyosarcoma. Ethical approval was obtained from Manchester, Salford and Trafford LREC to carry out a preliminary study. Glut-1 and VEGF protein expression were investigated in formalin-fixed, paraffin-embedded biopsies from two series of 20 patients each with neuroblastoma or rhabdomyosarcoma, and scored semi-quantitatively for staining area and intensity. Glut-1 was present in 10/20 cases of neuroblastoma, and 6/20 cases of rhabdomyosarcoma. VEGF staining was homogeneous in both tumour types, although staining intensity varied between tumours. Due to the small number of patients, trends, where observed, were non-significant. However, in neuroblastoma, borderline significant correlations existed between VEGF score and presence of Glut-1 ($r = 0.415$, $P = 0.077$, $n = 19$), and VEGF score and lack of survival ($r = 0.47$, $P = 0.042$, $n = 19$) (significance level $P = 0.05$), although there was no apparent correlation between presence of Glut-1 in either neuroblastoma ($r = 0.101$, $P = 0.673$, $n = 19$) or rhabdomyosarcoma ($r = 0.265$, $P = 0.265$, $n = 19$) and lack of survival. In neuroblastoma, non-significant trends existed whereby Glut-1 was associated with MYCN amplification ($r = 0.316$, $P = 0.201$, $n = 18$) and a trend also existed where both amplification of MYCN and Glut-1 positivity correlated with lack of survival ($r = 0.329$, $P = 0.183$, $n = 18$), where MYCN amplification alone showed no correlation ($r = 0.079$, $P = 0.755$, $n = 18$). Upon examination, virtually all tumours had large areas of abundant vascularisation. However, Glut-1 expression, if present, mostly occurred in patches of poor vascularisation around areas of necrosis, consistent with its regulation by hypoxia. In conclusion, Glut-1 expression may be an indicator of hypoxia in paediatric cancers, and may be mediated via both hypoxia-dependent mechanisms or through MYCN amplification. The detection of Glut-1 in paediatric cancers may highlight a role for novel hypoxia-selective or Glut-1-directed anticancer strategies.

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213
Attitudes of hospital doctors in paediatrics to spontaneous adverse drug reaction reporting

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Spontaneous reporting of suspected adverse drug reactions (ADRs) is the medical professions' major tool to detect and avoid further ADRs. Attitudinal studies have shown that there is a lower rate of reporting suspected ADRs by hospital doctors than by general practitioners, despite the fact that hospital doctors see more serious and severe reactions than those which occur in the primary care setting (Belton *et al* 1995; Belton 1997). There is an increasing interest in identifying, reporting and monitoring suspected ADRs in children; the extent of use of unlicensed and off label medicines in children is now widely recognized (Collier 1999). Children experience a different range of ADRs which are not necessarily predictable from the adult experiences. This is due to differences in morphology, metabolism, spectrum of disease and administered treatments (Gupta &Waldhauser 1997). This survey was developed to investigate the attitudes of hospital doctors to ADR reporting in children in Wales.

A self-administered questionnaire and letter of invitation was sent to all consultants and specialist registrars in paediatrics in Wales. The questionnaire addressed the issues that were believed to have a strong influence on ADR reporting by hospital doctors in paediatrics.

Of the 185 questionnaires mailed, only 43 (23.2%) were returned, 22 of these stated that they had reported at least one ADR during the last three years. Only 30% felt that all suspected ADRs to any medicines in children merit reporting despite the recommendation by the Committee on Safety of Medicines to do so. Over 95% responded that doctors have the ultimate responsibility to report a suspected ADR. Nearly 75% stated that they would not report a known complication of a medication. Educational measures, persistent reminders, involving other staff members, increasing the number of surveillance units, and financial incentives were few of the recommendations suggested by the respondents as to improve ADR reporting rate in children in Wales.

There is an urgent need to promote the Yellow Card Scheme and improve communication with hospital doctors. Proactive measures involving multiple interventions beginning at the level of undergraduate teaching and professional marketing of Yellow Card Scheme are required to include ADR reporting into the day to day clinical practice of hospital doctors.

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214
Acute effects of ibuprofen and its nitric oxide releasing prodrug on ulceration, erosion and nitric oxide synthase expression in rat gastric mucosa

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Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are effective in the treatment of pain and inflammation but are associated with gastrotoxicity. Efficacies of NSAIDs may relate to their ability to inhibit prostaglandin synthesis by alternative cyclooxygenase enzymes, which may compromise mucosal resistance to erosive effects of stomach acid. Modifications of NSAIDs to incorporate releasable nitric oxide (NO) are being explored (Wallace *et al* 1997). Three isoforms of nitric oxide synthase (NOS) can be identified in paraformaldehyde-fixed tissue by NADPH-diaphorase histochemistry. High levels of NO produced by the inducible NOS may contribute to tissue damage at the site of inflammation. Lower levels of NO expected from constitutive (neural and endothelial) isoforms may support gastro-protective functions, such as the inhibition of platelet aggregation and increased vascular perfusion (Nathan 1997). NSAIDs may modify endogenous NO synthesis and NO-conjugated prodrugs may substitute for the effects of endogenous NO. We therefore examined ibuprofen and its NO-prodrug (Ingram *et al* 2001) for gastrotoxic effects and association with endogenous levels of NOS. The effects of a single oral 4hr duration treatment with either ibuprofen or ibuprofen-NO (ea. 1.33×10^{-4}mol kg^{-1}) were compared with vehicle (aqueous Tween 80) using 16-h fasted male Wistar rats (~250 g; n = 5 per treatment). After treatment, stomachs were opened for counting of visible signs of ulceration then fixed and cryopreserved. Fifty micron thick sections underwent parallel histological processing for diaphorase histochemistry (Downing *et al* 1998) and morphometry by an observer blind to treatment, as follows: (a) Ulceration (by scaled visual score or as mean ulcers per cm of mucosa in section); (b) Erosion (mucosal thinning) and (c) NOS expression (mean % of section perimeter stained by diaphorase). Results are expressed as mean $\pm$ s.e.m. and the statistical significance of effects of treatments was evaluated by analysis of variance followed by Tukey's test.

Ibuprofen caused significant decrease in mean mucosal thickness (60.92 micron $\pm$ 0.66, $P < 0.001$) versus vehicle alone (77.37 micron $\pm$ 0.94). NO-ibuprofen also caused significant thinning although it showed significant sparing (64.71 micron $\pm$ 0.67, $P < 0.001$). Results were consistent with visible signs of erosion in the presence of ibuprofen and protection afforded by its NO-conjugate. However, mean numbers of ulcers measured per cm of mucosa did not resolve significant differences between treatments. Associated with its gastric sparing effects NO-ibuprofen treatment significantly ($P < 0.001$) elevated mean percent length of mucosa stained by diaphorase (28.15% $\pm$ 2.39) compared with ibuprofen (19.67% $\pm$ 1.76) and vehicle (18.52% $\pm$ 1.78). Results indicate a possible mechanism for gastro-protective effects of NO-ibuprofen.

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215
Acute reduction of nitric oxide synthase (NOS) in rat thymus without change in T-cellularity following oral non-steroidal anti-inflammatory drug (NSAID) treatments

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Conjugation of NSAIDs with esterase-releasable nitric oxide (NO) is being explored for its potential to reduce gastrotoxicity (Ingram *et al* 2001). Additional side effects include disruption of T-cell development within the thymus (Xu *et al* 2001) and the appearance of over active, possibly autoreactive, T-cells in the periphery (Yamamura *et al* 1996). Effects on thymic function of a single oral 4hr duration treatment with either indomethacin, indomethacin-NO (ea. 2×10^{-5} mol kg^{-1}), ibuprofen or ibuprofen-NO (ea. 1.33×10^{-4}mol kg^{-1}) were compared with vehicle (aqueous Tween 80) using 16-h fasted male Wistar rats (~250 g; n = 4 per treatment). Paraformaldehyde-fixed thymi were stored frozen (-20°C) before parallel processing for enzyme histochemistry. Two parameters of thymic function were measured from 100-micron sections: (i) Size of the cortex relative to total surface area gave the T-cellularity, an index of T-cell production or positive selection. (ii) While the abundance of medullary nitrergic cells (counts/mm^2) stained positive by NADPH-diaphorase was used as a marker of NOS. Negative selection (deletion) of potentially autoreactive T-cells is proposed to involve the induction of NOS within the thymic medulla and may therefore reflect quality control of T-cells. Relatively low levels of nitrergic cell expression in the presence of high T-cellularity have been associated with autoimmune susceptibility of the Lewis rat compared to Fischer strain (Downing *et al* 1998). Possible effects of NSAIDs on thymic organisation were compared using analysis of variance followed by Tukey's test and expressed as mean $\pm$ s.e.m. Both ibuprofen (57.08 $\pm$ 5.19; $P < 0.001$) and indomethacin (62.88 $\pm$ 4.84, $P < 0.001$) reduced nitrergic cell abundance compared with control (94.79 $\pm$ 6.70) in the absence of significant changes in T-cellularity. NO-conjugated drugs also suppressed the levels of NOS (NO-ibuprofen, 66.77 $\pm$ 5.23, $P < 0.001$; NO-indomethacin, 65.37 $\pm$ 6.55, $P < 0.001$) and also occurred in the absence of significant changes in T-cellularity. It is proposed that if the effects of acute oral treatment with either NO-conjugated or parent forms of ibuprofen or indomethacin are maintained over chronic periods of drug use they may pose a risk to effective immune tolerance and lead to the escape of autoreactive T-cells. A comparable autoimmune adverse drug reaction has been identified following withdrawal of the anti-inflammatory drug, cyclosporin A, which is also associated with inhibition of thymic nitric oxide synthesis (Kosaka *et al* 1990).

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216
Epidermal growth factor receptor tyrosine kinase plays an important role in the development of cardiovascular dysfunction in diabetes

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Diabetes mellitus is a complex metabolic disease caused by impairment of insulin signaling pathways and this disease can cause many chronic complications such as vascular disease, retinopathy, kidney disease, neuropathy and heart disease. It is likely that glucose and its metabolites mediate their adverse effects by altering various signal transduction pathways but not much is known about the role of receptor tyrosine kinases (RTKs). Here, we have examined the contribution of the epidermal growth factor receptor (EGFR) in the development of altered vascular sensitivity in the mesenteric bed of diabetic rats to the vasoactive hormone, noradrenaline.

Four groups of female Wistar rats were used: Group I; control rats were injected with the citrate buffer vehicle. Group II; diabetic rats without treatment; diabetes was induced by a single intraperitoneal (i.p) injection of 55 mg kg^{-1} streptozotocin (STZ). Group III; diabetic rats that received genistein, a broad inhibitor of RTKs, at a dose of 0.3 mg/200 g every other day for four weeks. Group IV; diabetic rats that received 0.3 mg/200 g of AG1478, a specific inhibitor of EGFR tyrosine kinase, every other day for four weeks. At the end of four weeks the rats were sacrificed and the mesenteric beds were isolated to measure changes in perfusion pressure in response to noradrenaline, NA (10, 100 and 1000 nmol). The level of EGFR tyrosine kinase activity was also determined biochemically by western blotting with antibodies specific for phosphorylated EGFR.

Hyperglycaemia persisted in the steptozotocin-treated rats and was 610 $\pm$ 24.4 mg dL^{-1} at four weeks as compared with 85.88 $\pm$ 5.0 mg dL^{-1} in the control rats. Four weeks after induction of diabetes significantly increased vascular reactivity to NA was observed in the mesenteric bed compared with the non-diabetic control rats (Table 1). Treatment of diabetic rats with genistein, a broad spectrum inhibitor of RTKs, normalized vascular reactivity to NA in the mesenteric bed. Similarly normalization was also observed with AG1478 (see Table 1). Biochemical analysis confirmed that EGFR tyrosine kinase activity was elevated in the mesenteric vasculature of diabetic rats and this elevation was normalized upon treatment with genistein or AG1478.

Table 1 NA-induced vasoconstriction in the perfused mesenteric bed

Group	Dose of NA	Mesenteric bed (mmHg)
I	10 nmol	39 $\pm$ 14
Control	100 nmol	97.5 $\pm$ 14.9
	1000 nmol	118.7 $\pm$ 8.8
II	10^{-8} M	85.7 $\pm$ 9.2
Diabetes	10^{-7} M	160 $\pm$ 15*
	10^{-6} M	172.5 $\pm$ 17.5*
III	10^{-7} M	97.7 $\pm$ 8.5**
Diabetes + genistein	10^{-6} M	119 $\pm$ 2.9**
IV	10^{-7} M	91.5 $\pm$ 8.5**
Diabetes + AG1478	10^{-6} M	114 $\pm$ 2.9**

Mean $\pm$ s.e. *$P < 0.05$ vs control; **$P < 0.05$ vs diabetes

Inhibition of the elevated EGFR tyrosine kinase activity in diabetes leads to improvement of cardiovascular reactivity in diabetic rats.

217
Nicotine protects primary rat mixed hippocampal/cortical cultures from NMDA-mediated toxicity: evidence for independent upregulation of nAChR α7 and neuroglia

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Recently, we have reported a profound upregulation of the nAChR α7 subunit protein following repetitive Cortical Spreading Depression (CSD)-induced preconditioning in the mouse (Chazot *et al* 2002). We hypothesised that this change may underlie in part the adaptive cytoprotection afforded by CSD. There is growing evidence that preconditioning can also be achieved with nicotine in a range of in-vitro cell culture systems. However, the mechanisms underlying preconditioning remains poorly understood. Interestingly, one common feature of nicotine-treatment is again a robust up-regulation of the nAChR α7 subtype. Here, we have utilised a protocol using a rat mixed cortical/hippocampal culture system to study nicotine preconditioning in-vitro with a view to investigate a potentially common mechanism of neuroprotection via the nAChR α7 subunit.

Primary cultures of mixed hippocampal/cortical neurons were prepared from hemispheres of 17–18 day-old rat embryos. Cells were plated onto poly-D-lysine-coated glass shards (200–500 000 cells/mL) in supplemented neurobasal medium (Verdon *et al* 2000). P14–21 cell cultures were subjected to the following treatments for 1 h at 37°C: (i) Exposure buffer solution (EBS) as described by Dajas-Bailador *et al* (2000); (ii) NMDA (200 μM) in EBS; (iii) NMDA (200 μM) + nicotine (10 μM) in EBS; (iv) Nicotine (10 μM) in EBS. The cultures were then returned to original culture media. Cell viability was assayed 20–24 h post treatments using an MTT assay (Carmichael *et al* 1987). FITCαBTX binding was performed using a standard protocol. Briefly, cell cultures were incubated with FITC-labelled αBTX at 3 μg mL^{-1} in PBS at 4°C for 15 min. Cell cultures were washed with PBS and then subjected to immunohistochemistry using a mouse anti-GFAP antibody (1% v/v), as described by Thompson *et al* (2002). Detection was achieved with an anti-mouse rhodamine-linked whole antibody (0.25%). Three independent replicates were performed for each experiment. Data are mean $\pm$ s.d., and analysed by Student's *t*-test with a significance level set at 0.05.

Exposure of 14–21 day old primary hippocampal/cortical cultures to NMDA for 1 h resulted in a 55 $\pm$ 14% reduction in cell viability 24 h post-treatment, in comparison with EBS control. Nicotine co-application conferred a significant protection to NMDA (19 $\pm$ 4% reduction in viability, in comparison with EBS control). Nicotine treatment alone had no significant effect upon cell viability. Exposure to nicotine elicited a significant increase in both FITCαBTX binding and GFAP immunoreactivity in comparison to controls (2.2 $\pm$ 1.0-fold and 2.3 $\pm$ 0.7-fold, respectively). Interestingly, there was very little overlap of fluorescence signals, indicating that each up-regulation effect occurred independently. Further work is underway to confirm this observation and probe the functional consequences of the nAChR α7 and GFAP upregulation.

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218
Actions of cryptolepine and the analogue, 2,7 dibromocryptolepine on mammalian intestinal muscle

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Cryptolepine is an indoloquinoline alkaloid which can be extracted from the west African climbing shrub *Cryptolepis sanguinolenta*. The agent has antibacterial and antiparasitic, including antiplasmodial, activity. A number of cryptolepine analogues, including 2,7 dibromocryptolepine, have been prepared and found to have potent activity against chloroquine-resistant strains of *Plasmodium falciparum* (Wright *et al* 2001). Decoctions of the plant are used in traditional medicine to treat malaria, hypertension and intestinal disorders, such as amoebiasis, but there is little information on its pharmacological actions. Previously cryptolepine has been reported to possess adrenoceptor antagonist and antimuscarinic properties (Rauwald *et al* 1992). In the present experiments we investigated the possible antimuscarinic actions of cryptolepine and compared these with the potent analogue 2,7 dibromocryptolepine, synthesised as described by Wright *et al* (2001).

Short lengths of ileum derived from Dunkin-Hartley guinea-pigs (500–800 g) were set up under 1 g tension at 32°C in Tyrode solution gassed with air and containing 100 μM hexamethonium bromide to antagonise potential nicotinic effects of the agents to be tested. Contractions of the longitudinal muscle of the ileum to carbachol (0.1–1.2 nM) were recorded isometrically. Following incubation for 20 min with cryptolepine (as hydrochloride, 10^{-7} to 10^{-4} M) or 2,7 dibromocryptolepine (as hydrochloride,10^{-7} to 10^{-5} M), concentration–response curves to carbachol were repeated. Cryptolepine and dibromocryptolepine were dissolved in distilled water: dilutions were freshly made daily.

Cryptolepine (10^{-7} M) caused no change in the carbachol concentration–response curve. Cryptolepine (10^{-6} M) slightly reduced maximal contraction, Emax decreased by 11.8 ± 4.8% ($P < 0.05$, N = 7). A further increase in concentration of cryptolepine (10^{-5} M) caused a greater reduction in maximal responses, Emax decreased by 23.0 ± 9.5% ($P < 0.05$, N = 5). No further reduction in response was elicited by an increased concentration of cryptolepine (10^{-4} M, N = 3). Administration of the analogue caused similar effects, 2,7 dibromocryptolepine (10^{-7} to 10^{-6} M) was without significant effect but dibromocryptolepine (10^{-5} M) reduced Emax by 19.0 ± 6.1 ($P < 0.05$, N = 4). Neither cryptolepine nor 2,7 dibromocryptolepine shifted the carbachol concentration–response curve to the right.

The results show that both the parent compound and analogue cause concentration-related reduction in carbachol-induced contraction of ileal longitudinal muscle. No evidence of an antimuscarinic action was observed in these experiments since there was no parallel rightward shift in the carbachol concentration–response curve. We conclude that cryptolepine and 2,7 dibromocryptolepine cause relaxation of intestinal smooth muscle by an action independent of muscarinic receptors.

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219
Insulin action on mammalian aorta is unmodified by changes in glucose concentration

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Insulin resistance and endothelial dysfunction are characteristics of type 2 diabetes mellitus. Diabetes confers heightened cardiovascular risk, type 2 diabetes patients being 2–4 times more likely than non-diabetics to develop cardiovascular disease (Sobel 2002). Prolonged hyperglycaemia may be an independent risk factor for development of diabetic vascular complications: strict blood glucose control both improves endothelial function and decreases microvascular disease (van Oostrom *et al* 2002). Hypoglycaemia elicits counter-regulatory responses including sympathetic activation which affects the cardiovascular system. Previously we confirmed that insulin relaxes rat aorta via endothelium-derived nitric oxide (NO). However, without endothelium, relaxation is lost and insulin potentiates contraction (McCurrie & Strati 2002). In this work we investigated effects of changing glucose concentration on insulin action in rat aorta.

Endothelium-intact or denuded aortic rings, prepared from Hooded-Lister rats (250–350 g) were placed under 2 g tension in euglycaemic Krebs' solution containing 11.1 mM glucose and 10 μM indomethacin (37°C, 95% O2, 5% CO2). Functional endothelium was confirmed by relaxation (>3 0%) to acetylcholine (1 μM) following contraction by KCl (60 mM). Concentration–response curves were constructed to KCl alone or following administration of physiological or pharmacological concentrations of bovine insulin (INS, 0.1, 1.0, 10 mU mL^{-1}) in intact or endothelium-denuded (ED) aortic rings. Experiments were repeated in Krebs' solution containing either 5.5 mM glucose (hypoglycaemic) or 44.4 mM glucose (hyperglycaemic Krebs'). Osmotic pressures were normalised by adding 5.5 mM sucrose to hypoglycaemic Krebs' and removing 33.3 mM NaCl from hyperglycaemic Krebs' solution.

In intact aorta maximal KCl-induced contraction was reduced by 8.8 ± 1.7, 10.2 ± 0.4, 19.7 ± 5.6% by INS, 0.1, 1.0 or 10.0 mU mL^{-1}, respectively ($P < 0.01$). In denuded aorta INS (0.1, 1.0, 10.0 mU mL^{-1}) potentiated maximal contractile responses to 115.9 ± 2.6, 125.3 ± 3.5, 136 ± 3.3% of control ($P < 0.01$). Neither hypoglycaemic nor hyperglycaemic Krebs' changed responses to KCl. Insulin (0.1, 1.0, 10.0 mU mL^{-1}) caused similar changes in contractile responses to KCl in both hypoglycaemic and hyperglycaemic Krebs': these were not significantly different from effects in euglycaemic Krebs' solution (Table 1)

Table 1 Comparison of effects of altered glucose concentration (5.5–44.4 mM) on modification by insulin (1–10 mU mL^{-1}) of maximal responses to KCl (100 mM, 100%) in normal Krebs'

Treatment		Intact	De-endothelialised
Hypoglycaemic Krebs'			
INS	0.1 mU	91.1 ± 3.9	120.4 ± 2.5
	1.0 mU	90.9 ± 5.1	122.5 ± 2.6
	10.0 mU	75.4 ± 2.1	128.1 ± 2.9
Hyperglycaemic Krebs'			
INS	0.1 mU	97.6 ± 1.1	117.2 ± 2.2
	1.0 mU	94.8 ± 3.5	123.1 ± 5.4
	10.0 mU	86.4 ± 2.4	129.4 ± 2.8

Results show that, in intact aorta, insulin administration similarly attenuated responses to KCl whether glucose concentration was normal, increased or decreased. Following endothelium removal, insulin potentiated contractile responses similarly at all glucose concentrations. In the short term, 2–3 h, no differences in insulin action were observed at different glucose concentrations. However, longer exposure of intact tissues to hyperglycaemia may be necessary to reveal evidence of decreased dilatation or deleterious endothelial changes associated with vascular disease in diabetes.

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220
Actions of potassium channel modulating agents on the peristaltic reflex of mouse colon

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Potassium (K^+) channels regulate excitability in smooth muscle membranes and are modulated by pharmacological agents of diverse chemical structure. Pinacidil and

cromakalim are known to open ATP and glibenclamide-sensitive channels (KATP channels), causing relaxation. There are few reports on gastrointestinal actions of these agents which may have potential in treating disorders of gastrointestinal hypermotility such as irritable bowel syndrome. Previously we characterised effects of KATP channel modulating agents in mouse ileum showing that relaxant effects of pinacidil and cromakalim are antagonised by glibenclamide (Yeung *et al* 2002). In this work we used a novel method to investigate effects of K^+ channel modulators on integrated peristaltic activity in mouse colon (Tuladhar & Naylor 2002).

Segments of proximal colon (3 cm in length, next to the caecum) were obtained from BKW mice (38–42g), cannulated at oral and anal ends and secured horizontally in a water-jacketed bath containing Krebs' solution at 37°C, gassed with 95% O2, 5% CO2. Regular peristalsis was achieved with intraluminal pressures of 2–4 cm of water. The volume ejected during each peristaltic wave, rate of peristalsis and tone of the tissue were measured as described previously (Tuladhar & Naylor 2002). Drugs were added cumulatively to the serosal side at 5–10 min intervals once regular peristalsis was obtained. Glibenclamide was equilibrated for at least 20 min before construction of concentration-response curves to pinacidil or cromakalim.

Cumulative addition of pinacidil (0.1–100 μM) and cromakalim (0.1–100 μM) caused concentration-related decreases in the volume ejected by each peristaltic wave without a significant effect on the rate of peristalsis. Ejection volume decreased from 0.42 ± 0.05 mL before addition of pinacidil to 0.06 ± 0.01 mL (n = 7) following the addition of the highest concentration (100 μM). Similarly ejection volume decreased from 0.42 ± 0.043 mL (n = 7) to 0.02 ± 0.038 mL (n = 7) with cromakalim (100 μM). Both compounds caused concentration-related reduction in the resting tone of tissues. The change in peristaltic rate from the beginning to the end of experiments was nonsignificant at 46.06 ± 3.45 and 40.78 ± 7.25 with pinacidil and 43.18 ± 2.88 and 32.03 ± 5.80 with cromakalim, respectively (n = 7, $P > 0.05$). Glibenclamide (0.1–1.0 μM, n = 14) alone had no significant effect on volume ejected, tone or rate of peristalsis. Furthermore, concentration response curves to pinacidil and cromakalin were not significantly altered by glibenclamide (1 μM, n = 7).

The results showed that cromakalim and pinacidil both decrease the efficiency of the peristaltic reflex. Selective reduction in tone and volume ejected without effects on peristaltic rate suggests that these compounds are unlikely to act on the neuronal circuitry of peristalsis, but possibly produce their effects by relaxation of the effector circular smooth muscles. Interestingly, actions of both pinacidil and cromakalim were unaffected by glibenclamide, indicating that, unlike longitudinal muscles of mouse ileum, the relaxant effect of these compounds in mouse colon is mediated by a glibenclamide-insensitive mechanism.

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221
Evidence for a complex interaction between haloperidol and glycine in the modulation of dopamine release in the rat striatum

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The clinical efficacy of classical antipsychotics strongly correlates to their ability to block dopamine D_2 receptors. This observation lead to the development of the dopamine hypothesis of schizophrenia, that suggested that some of the symptoms of schizophrenia were produced by an over-activity of dopaminergic neurotransmission. It is known that the antipsychotic haloperidol is an effective treatment for the positive symptoms of schizophrenia and recent work by Heresco-Levy (2000) shows that adjunctive treatment with the amino acid glycine improves symptom control. In the current study the combined and individual effects of acute haloperidol and glycine were investigated on dopamine release from isolated rat striatum.

Striata from adult male Sprague-Dawley rats were rapidly dissected out and sliced into prisms (350 μm), washed and suspended in Krebs-bicarbonate buffer (composition in mM: NaCl 125, KCl 2.5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 10, pH 7.4) for 30 min to recover at 37°C. Then, 5-min samples of incubation media were collected and analysed for dopamine content by HPLC. At t = 15 and t = 50 min, a 30 mM potassium buffer was applied for 5 min. 10 μM haloperidol and 1 mM glycine, alone and in combination, were applied by bath application from t = 30 onwards. The mean dopamine content of samples collected prior to the first potassium stimulation was used to normalise all subsequent values. The two KCl-induced stimulation were compared using a paired t-test with significance if $P < 0.05$. In control conditions (no drugs), the two stimulations of the tissue with 30 mM KCl produce an identical robust increase in dopamine release. When applied separately, both glycine and haloperidol induced a significant increase (64 ± 42% n = 6, 61 ± 39% n = 5) in the potassium-evoked release of dopamine relative to the first stimulation. However, when applied in combination, potassium-evoked release of dopamine decreases by 30% (± 26, n = 6). The increase in potassium-stimulated effects with haloperidol is likely due to a loss of feedback control due to autoreceptor D_2 antagonism. The effects of glycine may have been produced by an increase in glutamatergic activity due to action at the NMDA receptors' $glycine_B$ site. The combination effect may arise because the glycine effect is mediated through NMDA receptors, which the haloperidol antagonises, leading to a loss of this effect and the possible unmasking of an inhibitory effect of glycine mediated via the strychnine-sensitive glycine inhibitory receptor.

Only haloperidol alone had any significant effect on spontaneous dopamine release, producing a 200% (± 79, n = 5) increase over baseline; evidence that D_2 receptors play an important role in control of spontaneous dopamine release.

These results suggest the existence of a complex interaction between haloperidol and glycine on the regulation of striatal dopamine neurotransmission. That the beneficial therapeutic effect of glycine co-administration has its basis by preventing the enhancing effects of haloperidol on dopamine release in stimulated conditions is an interesting possibility.

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222
A physiological role for P2X7 receptors at the rat skeletal neuromuscular junction in-vitro

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Immunocytochemical evidence suggests that P2X7 receptors (a novel class of ligand-gated cation channels), in addition to mediating fast excitatory neurotransmission in CNS, may be located presynaptically on nerve terminals in the peripheral nervous system where they may modulate transmitter release (Khakh & Henderson 2000).

Deuchars *et al* (2001) have identified P2X7 receptors on mammalian nerve terminals and cell culture studies suggest depression of excitatory post-synaptic potentials (EPSPs) after agonist binding (Knight *et al*, Soc for Neurosci abstract booklet, Orlando, 2000). The current study examined whether P2X7 receptors on rat motor nerve terminals play a functional role when receptor reserve is eroded pharmacologically.

Phrenic nerve-hemidiaphragms were isolated from male Wistar rats (250–350 g) that had been humanely killed. Preparations were maintained under isometric conditions in aerated McEwens solution (pH 7.4) at 32°C. The nerve was stimulated using 0.2 ms pulses of twice maximal voltage (typically 10 V) at 0.1 Hz. Tetani were delivered at 50 Hz for 2 s every 5 min. Tetanic fade was calculated by standard methods (Gibb & Marshall 1986). Student's *t*-test (two-tailed analysis) was performed on paired data. All other data were subjected to analysis of variance followed by the Tukey-Kramer multiple comparisons test. Results are expressed as mean ± s.e.m.

Pilot studies showed that the application of either 1300 μM ATP or Brilliant Blue G (BBG, 100 μM) did not alter twitch tension under physiological conditions. A

25 min application of tubocurarine (4.5×10^{-7} M; n = 31) eroded twitch tension to 83 ± 3% of the control ($P < 0.001$). Subsequent administration of ATP (1300 μM, n = 10) resulted in a further decrease to 54 ± 5% ($P < 0.001$). Application of BBG (100 μM, n = 6) significantly ($P < 0.05$) augmented the twitch response in the presence of 4.5×10^{-7} M tubocurarine (circa 16%). This dose of tubocurarine produced a tetanic fade of 63 ± 0.04% (n = 12) and this fade was enhanced to 93 ± 0.01% in the presence of ATP (1300 μM; n = 12) but was reduced to 0.04 ± 0.02% in the presence of 100 μM BBG (n = 7). The effects of the P2X7 receptor modulators on tetanic fade were highly significant ($P < 0.0001$).

In the presence of tubocurarine, P2X7 agonists appear to depress tension whilst antagonists potentiate neuromuscular transmission. The profound effects on the tetanic responses support the suggestion that these receptors are presynaptically located.

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223
Role of nitric oxide on oestrogen-induced relaxation in different blood vessels of the rat

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Epidemiological studies indicate that oestrogens in Hormone Replacement Therapy protect postmenopausal women against cardiovascular disease. However, the mechanism remains controversial: involvement of nitric oxide, changes in calcium mobilisation, enzymes and ion channels have been proposed. Miller *et al* (2002) reported that oestradiol relaxed both isolated arteries and veins via released nitric oxide (NO) whereas Prackash *et al*(1999) observed that arterial relaxation involved calcium influx inhibition and facilitation of calcium efflux. One explanation for the different mechanisms proposed may be use of vessels from different vascular beds. This work compares the contribution of NO to oestradiol-induced relaxation of rat aorta and portal vein (PV).

Aortic rings with or without endothelium from male Hooded-Lister rats (250–350 g) were placed in Krebs' solution containing 10 μM indomethacin (37°C, 95%O_2, 5% CO_2) (KS) under 2 g tension. Functional endothelium was confirmed by relaxation (> 30%) to acetylcholine (1 μM) following contraction by KCl (60 mM). Portal veins were studied in KS under 0.5 g tension. Concentration–response curves constructed to KCl (10–100 mM) in each tissue were repeated after administering 17β-oestradiol (EST, 10–20 μM). Following 20 min incubation with L-NAME (100 μM), an NO synthase inhibitor, EST was retested.

EST similarly relaxed contraction of intact aorta and PV, being less effective in de-endothelialised aorta (Table 1). Administration of L-NAME did not affect relaxation in portal vein or de-endothelialised aorta but greatly reduced relaxation of intact aorta. This was reversed by L arginine (1 mM), the substrate for NO production (Table 1). L arginine did not affect relaxation in PV or denuded rings.

Table 1 Effect of modifying nitric oxide production on relaxation by oestradiol (10 μM)

Drug	Portal vein	Intact aorta	Denuded aorta
EST alone	50.6 ± 5.7	51.7 ± 4.6	28.1 ± 7.3
+ L-NAME	44.6 ± 8.2	24.1 ± 3.7*	22.7 ± 7.6
+ L arginine	47.9 ± 8.0	42.7 ± 7.6#	39.5 ± 5.1

Numbers represent % reduction in maximal contractile response.
* $P < 0.01$, #$P < 0.05$, compared with L-NAME (Student's paired *t* test)

Incubation of tissues with ICI 182 780 (10 μM), a specific oestrogen receptor antagonist, prior to administering EST (10 μM), did not affect relaxation.

Results show that oestradiol action in portal vein is independent of NO production, while in intact aorta endothelium-derived NO appears to contribute significantly to relaxation. The lack of effect of ICI 182 780 indicates that classical oestrogen receptors are not involved in oestrogen-induced relaxation. In previous work on portal vein, which, unlike aorta, exclusively depends on extracellular calcium for activation, actions of oestradiol appeared to involve altered calcium mobilisation (Al-Hawadi & McCurrie 1997). Oestradiol has many actions on blood vessels: major factors determining mechanisms of relaxation in different vessels are likely to be excitation processes and the contribution of endothelium in modulating muscle responses.

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