175P TRANSIENT HISTAMINE H_1-RECEPTOR MEDIATED AGONIST RESPONSES MAY YIELD ERRONEOUS pK_A ESTIMATES: APPLICATION OF EQUILIBRIUM AND KINETICS MODELS OF AGONIST ACTION

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In this study we determined the pK_A value of histamine (HA) at H_1-receptors in two functional *in vitro* guinea pig (male Dunken Hartley, 250-500g) bioassays: gallbladder (gb) and gastric muscle (gm) (Watt *et al.*, 1995). In both assays HA responses faded, albeit with different characteristics. The responses to a ~90% maximally effective HA concentration (1μM) in gm reached a peak at 16.6±3.1 min (n=6), which faded slowly towards baseline (% fade (n=6): 60 min 13.7±2.9; 120 min, 30.4±6.3). However, in the gb, the responses reached a peak within 6.7±1.2 min ([HA]=10μM, n=6) and faded faster initially achieving a steady state level (% fade (n=6): 60 min, 59.2±5.7; 120 min, 57.8±6.8). Over the same time period the muscarinic receptor agonist, 5-methylfurmethide, produced stable responses in both assays. We interpret the HA response fade to be due to receptor desensitisation rather than non-specific tissue fatigue.

In both assays cumulative HA concentration response (E/[A]) curves were constructed by incremental rapid dosing at the peak response (E/[A] curve duration: gb, ~12 min & gm, ~40 min). Additionally it was also possible to construct a more prolonged (~280 min) cumulative E/[A] curve in the gb by incremental dosing at the steady state response level. These E/[A] curves were generated following progressive degrees of receptor alkylation with PBZ (0.1-10μM with 5 min tissue exposure) and the data analysed using an equilibrium model (Black & Leff, 1983) to obtain *apparent* pK_A values ($pK_{A(app.)}$). Replicate experiments using the rapid E/[A] curve approach gave similar results (gb; 5.09±0.48 & 4.89±0.27: gm; 5.66±0.44, 5.76±0.34 & 5.71±0.41) and thus were pooled. The $pK_{A(app)}$ values in the gb were ~0.7 log units lower than the gm ($P<0.01$). Furthermore, for the gb the $pK_{A(app)}$ estimate was ~1.5 log units higher when using the prolonged rather than rapid E/[A] curve and so does not lie within the 95% confidence interval for the rapid E/[A] curve $pK_{A(app)}$ value. Although ambiguity surrounds pK_A values, they have proven useful in differentiating receptor subtypes. Superficially, these $pK_{A(app)}$ differences may indicate different HA receptor subtypes. However, the phenomena of receptor inactivation, manifested as fade, has been reported to result in an underestimation of pK_A values (Leff, 1986) but this model could not account for the fade characteristics in this study. Therefore the simplest model (Lew *et al.*, 2000), which incorporates both receptor inactivation and reactivation, was employed to analyse the time-response profiles and re-interpret the receptor alkylation data sets. The resulting simulations provided good qualitative and quantitative description of the experimental data without the need to invoke differences in the pK_A values (i.e. 5.5, derived from ratio of k_{-1} to k_1) between assays and experimental designs. However, the rates of receptor inactivation and reactivation differed between assays. This pK_A value is similar to the $pK_{A(app)}$ estimate obtained from the equilibrium model fit of the gm data (i.e. 5.7) and is probably an indication that the extent of fade reflects a small degree of receptor inactivation.

These approaches demonstrate how a kinetic model of receptor inactivation with reactivation can be used to avoid potential pitfalls of erroneous agonist pK_A determinations that can arise when equilibrium models are employed to analyse data with underlying fade.

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176P COMPARISON OF TNF-α AND IL-1β MEDIATED IL-6 PRODUCTION IN A NOVEL HUMAN RENAL CO-CULTURE PERFUSION SYSTEM

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Current clinically utilised cytokines are species specific. Animal based models are not ideal for testing species specific effects, while alternative *in vitro* cell culture models fail to closely mimic the *in vivo* state. In order to address these limitations a novel perfusion system was developed to allow for testing of potential side effects of human recombinant cytokines. A new cell culture apparatus allowing for permanent and continuous administration of nutrients, continuous removal of metabolic wastes and continuous administration of test compounds was designed. The apparatus allows for different cell types to be cultured in separate compartments within the same chamber allowing for non-contact cell-cell interaction while exposed to perfusion conditions.

Microporous membranes (0.2μm pore size) were placed in the test chambers and coated with rat tail collagen (2μg/cm^2). Human proximal tubular epithelial cells (HK-2) were cultured in the apical compartment, while human microvascular endothelial cells (5A32) were cultured in the basolateral compartment either in monoculture or non-contact coculture. Cells were cultured in 1% FCS, MCDB 131, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin. Culture conditions were maintained at 37°C with 95% Air/ 5% CO_2.

Cells were allowed to achieve confluence and acclimatise to perfusion conditions (perfusion rate 1ml / hour) for 24 hours. Cells were treated on both the apical and basolateral side with TNF-α (1 ng/ml) or IL-1β (1 ng/ml) for up to 48 hours. Cell culture medium was removed at 4, 24 and 48 hours and assayed for IL-6 production by ELISA. Morphological examination of the cells was also observed by phase contrast microscopy.

Basal production of IL-6 in HK-2 cells at 4, 24, 48 hours (apical release 81 ± 28, 136 ± 65, 163 ± 63 pg/ml), (basolateral release 68 ± 27, 78 ± 51, 68 ± 25 pg/ml) was high in comparison to 5A32 cells (levels undetectable). Co-cultured HK_2 and 5A32 cells showed basal IL-6 production at 4, 24, 48 hours (apical release 168 ± 19, 183 ± 29, 197 ± 30 pg/ml), (basolateral release 83 ± 5, 87 ± 2, 75 ± 16 pg/ml). TNF-α (1ng/ml) stimulated production of IL-6 in HK-2 and 5A32 co-culture at 4, 24, 48 hours (apical release 450 ± 142, 460 ± 47, 394 ± 33 pg/ml), (basolateral release 633 ± 199, 811 ± 61, 808 ± 151 pg/ml). IL-1β (1ng/ml) stimulated production of IL-6 from HK-2 cells at 4, 24, 48 hours (apical release 1706 ± 446, 4171 ± 599, 3991 ± 740 pg/ml). IL-1β stimulated co-cultures of HK_2 and 5A32 cells at 4, 24, 48 hours also produced IL-6 (apical 1492 ± 21, 2295 ± 591, 3024 ± 667 pg/ml), (basolateral 2097 ± 348, 1986 ± 623, 1449 ± 337 pg/ml).

TNF-α and IL-1β stimulated Il-6 release from HK-2 and 5A32 cells. A novel perfusion system that facilitates co-culture of different renal cell types and continuous monitoring of cellular responses was developed.

177P ROBUST AND SIMPLE SOFTWARE / HARDWARE TO RECORD MUSCLE CONTRACTIONS IN THE PRACTICAL CLASS

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The kymograph is simple to use but lacks precision and sensitivity offered by mechanical transducers. Commercial systems which might replace the kymograph target the researcher rather than the student. Our objective was to create a recording system which is intuitive and easy to run in a busy practical class.

Hardware: The hardware consists of a robust almost unbreakable piezoelectric transducer, a bridge amplifier, an interface card (Data Translation DT302) and computer.

Software: The software displays the record on the computer screen as well as controlling the gain, acquisition rate and zero offset. On opening the application, it self zeroes the input, and begins the data acquisition. It runs on 20 work-stations each running under Windows 95/98 and networked with Novell. The initial settings are can be configured on one computer and these settings are read by the other machines on start up from a configuration file on the server. This file also contains a preset list of protocols, and stores other settings which allow data saving and replay, facilities that are password protected.

Security features: To obtain a hard copy, each student at a work-station has to enter their name on the screen and then one copy is printed out for each name. To ensure that the record used for the write-up is the student's own work, the printout is date stamped and shows the student name. The printout also shows event marks and user entered text describing the protocol.

Help: The software comes with a help file explaining the function of the controls, information related to the experiments and a video file showing the dissection and installation of the preparation.

Usage: The system has been exposed to around 2000 students during the past two years. The students range in ability from diploma nursing to second year science students. It has been used to rabbit gut contractions, frog heart, frog gastrocnemius muscle and vitalograph recordings for mostly 2 hr classes.

178P FOLATE-MEDIATED DELIVERY OF THERAPEUTIC AND IMAGING AGENTS TO CANCER TISSUE *IN VIVO*

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The vitamin folic acid (FA) enters cells either through a carrier protein (termed the reduced folate carrier) or via receptor-mediated endocytosis facilitated by the folate receptor (FR). Because folate-drug conjugates are not substrates of the former, they penetrate cells exclusively via FR-mediated endocytosis. Overexpression of FR on cancer cells allows tumour-selective target-ing of folate-drug conjugates to malignant tissues, including cancers of the ovary, cervix, endometrium, kidney, breast, brain, lung and colon. FR expression appears to be even further upregulated in cancers that are resistant to standard chemotherapy in higher grade and later stage neoplasms. Thus, precisely those cancers that are most difficult to treat by classical methods are most easily targeted by folate-mediated therapy.

Folate-mediated tumor targeting has been exploited to date for delivery of the following molecules and molecular complexes: (i) protein toxins; (ii) chemotherapeutic agents; (iii) genes; (iv) oligonuceotides; (v) ribo-zymes; (vi) radioimaging agents; (vii) MRI contrast agents; (viii) liposomes with entrapped drugs; (ix) radiotherapeutic agents; (x) immunotherapeutic agents; and (xi) enzyme constructs for prodrug therapy. In all cases, in vitro studies demonstrate a significant improvement in potency and cancer cell specificity over the non-targeted form of the same agent. Where live animal studies have been conducted, they also reveal improved response rates. Results of both preclinical and clinical studies of several folate-targeted drugs will be presented.

Recent applications of folate targeting to the diagnosis and treatment of various inflam-matory diseases will also be described. During clinical evaluation of our folate-targeted imaging agent, it was observed that arthritic (but not normal) joints, as well as malignant issue, were readily visualised. We have subsequently learned that macro-phages express the folate receptor, and that the receptor is unable to bind either folic acid or its drug conjugates until the macrophage is activated. As a consequence, normal macrophages are refractory to folate-drug conjugates, but activated macrophages take them up avidly.

Based on studies in animals, we envision applications of folate targeting in the treat-ment of diseases caused by activated (but not resting) macrophages, including rheuma-toid arthritis, ulcerative colitis, Crohn's disease, psoriasis, osteo-myelitis, multiple sclerosis, graft versus host disease (e.g. organ transplant rejection) and athero-sclerosis.

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