361P MASTER OF RESEARCH (M.Res) IN BIOLOGICAL SCIENCES: THE MANCHESTER EXPERIENCE

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The changing nature of undergraduate education has led to a widely-held perception that studies to B.Sc. level no longer prepare a student with the appropriate skills to succeed as a biological scientist either within industry or as a direct prelude to Ph.D study. The concept of M.Res. was developed through consultations with the Office of Science and Technology and was aimed to satisfy the perceived need for graduates with a wider appreciation of the working environment and more research skills. In 1995-96 the School of Biological Sciences, University of Manchester (amongst 13 centres nationwide for BBSRC and MRC pilot schemes) ran its M.Res. programme for the first time.

In designing our programme we consulted widely with over 400 key members of the industrial community. Industrial input continues as the course develops with an Industrial Advisory Group, industrial contribution to taught elements of the programme, staff seconded from industry to take the M.Res. programme and the opportunity for research projects to be performed in an industrial environment. The M.Res is extremely important with respect to the training, and re-training, of pharmacologists and other specialists for the pharmaceutical industry and other biomedical research.

The key elements of the course are

- a rotation through three research projects that can take place either within academia or industry. The research projects occupy approximately 60% of the student's time,
- comprehensive modules to develop personal and professional transferable skills and basic research skills,
- taught modules to develop specialist theoretical knowledge,
- research training modules to introduce students to a range of techniques they may not encounter in their research projects,
- instruction in legislative matters such as would apply to the use of animals under a Home Office licence or genetically-manipulated organisms
- training in key skills such as computation, statistics, experimental design

We maintain a high level of flexibility in defining the details of these components to ensure that the training programme is tailored to the individual needs and aspirations of the students.
The M.Res. programme framework permits students to receive training in any of one of the sixteen areas of research strength defined in the School from Cell Biology to Pharmacology. Taught elements are assessed by examination and the final thesis incorporates the work of all three research projects.

The programme is now in its third year and, in all, we have admitted 83 students to the programme. 32 of these were supported by research councils, 12 by industry and the remainder were self-funded or supported in part by the European Social Fund.

Our limited outcome data (year 1 only) show that approximately a third of the students enter industry directly from M.Res. The remainder have continued training towards Ph.D. level. Our experience to date indicates that the M.Res. attracts very high quality applicants and delivers training which is valued by students and employers and is a very effective medium for technology transfer from university to industry.

362P DESIGNING LIGANDS FOR G PROTEIN-COUPLED RECEPTORS: GROWTH HORMONE SECRETAGOGUES - A CASE HISTORY

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In recent years, growth hormone releasing peptides (for e.g. GHRP-6; His-DTrp-Ala-Trp-D-Phe-LysNH_2) and peptidomimetics have received considerable attention as potential alternatives to injectable growth hormone (GH) replacement therapy. GH secretagogues present a number of opportunities for therapeutic intervention since these compounds can restore and enhance pulsatile GH secretion in humans.

The design of these peptidomimetics is interesting since they are agonists. Furthermore, their discovery was achieved without knowledge of the molecular target and without structural information concerning the endogenous hormone that they presumably mimic. We have disclosed an orally active GH secretagogue, MK-0677, that is currently in clinical trials. Only recently with the aid of peptidomimetic ligands that were derived from MK-0677 has the receptor for the secretagogues (GHS-R) been identified, characterized and cloned. The natural ligand has still not been identified. Therefore, the GHS-R is an orphan receptor.

This presentation will discuss the design and biological activities of MK-0677 and a series of novel probes that were useful in characterizing the receptor and in unravelling the cellular mechanisms that ultimately trigger GH release.

Finally, results from site-directed mutagenesis and molecular modeling studies will be presented that provide insights in to some of the structural requirements for activating the human GHS-R.

363P 'ORPHAN' G PROTEIN-COUPLED RECEPTORS: THE NEXT GENERATION OF DRUG TARGETS

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The pharmaceutical industry has embraced genomics to provide it with new targets for drug discovery. Large scale DNA sequencing has allowed the identification of a plethora of DNA sequences distantly related to known G protein-coupled receptors (GPCRs), a superfamily of receptors that have a proven history of being excellent therapeutic targets.

In most cases the extent of sequence homology is insufficient to assign these 'orphan' receptors to a particular receptor subfamily. Consequently, reverse molecular pharmacological and functional genomics strategies are being employed to identify the activating ligands of the cloned receptors.

Briefly, the reverse molecular pharmacological methodology includes cloning and expression of orphan GPCRs in mamalian cells and screening these cells for a functional response to cognate or surrogate agonists present in biological extract preparations, peptide libraries, and complex compound collections.

The functional genomics approach involves the use of a 'humanized' yeast cell, where the yeast GPCR transduction system is engineered to permit functional expression and coupling of human GPCRs to the endogenous signalling machinery.

This system provides an excellent platform for rapidly screening for receptor agonists. Once activating ligands are identified they can be used as pharmacological tools to explore receptor function and relationship to disease.

364P ORPHAN RECEPTORS: HOW MANY UNKNOWN MESSENGERS?

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More and more orphan G protein-coupled receptors have been made available by various cloning procedures, such as PCR amplification using degenerate oligonucleotides, or systematic sequencing of cDNA libraries. These orphan receptors potentially constitute elements of fundamental communication pathways in various systems. We have demonstrated that such orphan receptors could be used as part of a bioassay in order to isolate the cognate ligand from tissue extracts, and characterize the receptor function and pharmacology.

The human orphan receptor ORL1, related to opioid receptors, was expressed in a CHO cell line, and a novel heptadecapeptide, presenting weak similarities with dynorphin A, was identified and purified on the basis of its ability to inhibit cAMP accumulation. This peptide was shown to increase sensitivity to pain in a hot plate assay, and was named nociceptin, while the structure of the cDNA and gene encoding the peptide precursor was analyzed. This was the first time that a novel bioactive peptide was isolated on the basis of its activity on an orphan receptor.

We are currently working on a series of orphan receptors expressed in the central nervous system, and for which the intracellular cascade is not known. General procedures allowing us to prepare peptide extracts from brain, separate them by HPLC and assay the activity of the fractions on cell lines expressing various receptors have been established. Model systems and preliminary data obtained for orphan receptors will be presented.

365P THE CGR1P RECEPTOR REQUIRES A SINGLE TRANSMEMBRANE PROTEIN FOR FUNCTIONAL EXPRESSION

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Calcitonin gene related peptide (CGRP) is a neuropeptide with many activities that are thought to be mediated by seven transmembrane G protein coupled receptors (7TMs).

A likely candidate for the CGRP receptor, the calcitonin receptor-like receptor (CRLR), is only activated by CGRP in some cell backgrounds. From SK-N-MC cells we have expression cloned a novel 148 amino acid protein, Receptor Activity Modifying Protein (RAMP1), for its ability to potentiate the endogenous CGRP receptor in oocytes from *Xenopus laevis*. Although not a receptor itself, RAMP1 appears to be essential for CRLR to function as a CGRP receptor in mammalian cells. Transient or stable co-expression of CRLR and RAMP1 produced cells that both responded to CGRP by increasing intracellular cAMP and expressed a CGRP receptor indistinguishable from that native to SK-N-MC cells. CRLR or RAMP1 alone had no activity.

Stable Swiss3T3 cell lines were created in which the CGRP receptor signalled to pertussis toxin sensitive and insensitive G proteins. In *Xenopus* oocytes pertussis toxin sensitive signalling was constitutive, occurring in the absence of CGRP. Co-expression of CRLR and RAMP1 leads to both proteins being expressed at the plasma membrane and to an increase in the molecular weight of CRLR from 58 kDa to a mature, endoglycosidase H resistant, form >66 kDa. In intact cells it is this 66 kDa form which cross-links to [^{125}I] human αCGRP.

The requirement for two proteins to functionally express a CGRP receptor can explain its resistance to expression cloning and the failure of CRLR to function in some cell lines. This type of regulation may also occur for other 7TM receptors.

366P GPCR-G PROTEIN FUSIONS: A NOVEL STRATEGY TO EXAMINE SIGNAL TRANSDUCTION THROUGH SPECIFIC RECEPTOR-G PROTEIN TANDEMS

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The interactions of G protein-coupled receptors (GPCRs) and G proteins are regularly examined following transfec-tion of relevant cDNAs into mammalian or insect cell lines. Often, however, little attention is given to the ratios of expression of these polypeptides, their relative cellular distribution and how these may affect their interactions, signal transduction properties and detailed pharmacology.

As a novel approach to define both that the stoichiometry of expression of the GPCR and G protein of interest is 1:1 and that they will be in proximity following expression, we have constructed a series of fusion proteins in which the N-terminus of a G protein a subunit is linked directly to the C-terminal tail of a receptor. This allows expression of a single polypeptide which has the functions of both GPCR and G protein.

Using the α_{2A}-adrenoceptor linked to the α subunit of G_{i1} as a model, I will demonstrate how such fusion proteins can be used to:

(1) measure the turnover number of the G protein in response to binding of an agonist ligand to the receptor;

(2) provide efficacy measurements for ligands at specific GPCR-G protein tandems;

(3) assess the role of co-and post- translational acylation of G proteins in signal transduction;

(4) demonstrate the role of G protein bg complexes in ternary complex formation;

(5) make quantitative measurements of the effects of minor mutations on GPCR-G protein interactions;

(6) examine whether agonists can selectively "channel" signals through GPCR tandems containing closely related G protein a subunits.

367P SCREENING ASSAYS FOR G PROTEIN-COUPLED EVENTS

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High throughput screening for therapeutically active compounds initially focused on identifying ligands which interacted with receptor binding sites. This type of receptor binding assay gave information on binding but did not classify compounds as either agonists or antagonists. The use of an assay system to measure GTP binding in cell membranes enables those compounds which exert an agonist effect to be identified at the first screening step.

SPA is an homogeneous assay format which does not require separation of bound from free radioactivity. It can be used to measure GTP binding in cell membranes, containing G protein coupled receptors, by binding membrane fragments to wheat germ agglutinin-coated SPA beads. When the membranes are treated with an effector compound, in the presence of [^{35}S]GTPγS, a non-hydrolysable form of GTP, only the [^{35}S]GTPγS bound to the membrane, and hence, to the bead will be detected in the assay. The lack of a separation step makes this assay format ideally suited for high throughput applications.

A further refinement would be to look at a downstream event which is a result of G protein activation and which requires the use of intact and fully functioning cells. One example of this type of assay is the measurement of arachidonic acid release from the membranes of cells by phospholipase A2 which has been activated via a G protein.

This can also be done in a homogeneous assay format using Cytostar-T scintillating microplates. A cell monolayer is grown on the base of the 96 well microplate, labelled *in situ* with [^{14}C]arachidonic acid and then treated with an effector. Arachidonic acid release can then be measured directly, in real time, by the reduction in the microplate signal. This 'one plate' format of this assay also makes it more suited for higher throughput applications than conventional sampling assays.

368P TEACHING PHARMACOLOGY IN THE 21st CENTURY: WHAT WILL THE CUSTOMERS BE LIKE?

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The terms customer and higher education (HE) are not often associated but various groups might be considered as customers for HE.

One customer group is the students. Mature students who often pay their own way have always been vocal about the shortcomings of their HE experience and now Dearing has recommended a loan based HE including a contribution to fees "customer satisfaction" is likely to take a higher profile. "Branding", "packaging", "customer friendly service", "value for money" and "the sales environment" are terms common in the retail sector and have clear counterparts in HE. Competition for numbers of students and for quality of intake is already with us especially in the medical field. Dearing has recommended increasing participitation rates to 40% and this will further increase diversity of ability and motivation in pharmacology students which has implications for course design.

The government pays HE for educational provision. This customer is exerting a stronger influence on HE not only in numbers/price of students admitted but also in terms of the content, quality, style and objectives of the teaching which is acceptable. There is no question but that the unit of resource has dropped sharply over the last few years. There have been a number of initiatives, primarily through the Department for Education and Employment, changing the nature of the teaching in HE, the material taught and the emphasis on the purpose for which HE is provided. Enterprise in Higher Education, the Higher Education Discipline Networks and Teaching Quality Assessment all effectively represent customer pressure on HE.

Employers are customers who may choose to buy the products of HE. The majority of pharmacology student enter occupations involving the pharmaceutical industry or study for a higher degree but a substantial and increasing minority enter non-pharmacology areas. Both types of employer are quite clear that subject specific knowledge is of less importance that other generic skills and attitudes in graduates they choose to employ and this will continue to affected HE courses. Increasingly employers are obtaining input into the provision of HE through course committees, government initiatives, and the increasing emphasis placed on the importance of work experience.

Professional bodies could be seen as customers. Some have as yet little customer power, for example the BPS, while others, like the GMC, have a great deal. The latter has been a notable catalyst for change in medical pharmacology courses over the last 5 years.

A fifth customer is the institution in which the course is provided. Does the course break even financially or is it subsidised by other courses and to what extent? This question was never asked of the excellent pharmacology course which I and the other student (singular) in my year experienced.

As with any organisation trying to survive in a marketplace - and I am firmly convinced pharmacology providers are in a market place - the trick is to keep all the customers happy all the time. There is undoubtably a variety of disparate solutions to this aspiration and it seems likely that pharmacology courses across the UK will increasingly reflect a diversity of response to pressures from our different customers as we enter the millennium.