458P CHARACTERISATION OF PHOSPHATIDIC ACID PHOSPHOHYDROLASE-2 IN AIRWAY SMOOTH MUSCLE

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Phosphatidic acid (PA) has been shown to have a number of intracellular functions. A potential role for PA in mitogenesis has also been proposed (Khan *et al.*,1994;Fukami *et al.*,1992). In guinea-pig airway smooth muscle cells (GPASM) PA can be derived from phosphatidylcholine (PC) hydrolysis via phospholipase C (PLC) and phospholipase D (PLD) in response to a variety of agonists, including Bradykinin and PDGF. In bradykinin stimulated GPASM cells PLD-derived PA appears to be upstream of cAMP formation which, in these cells, is anti-mitogenic (Stevens *et al* ., 1994). Thus, regulation of the PA concentration within these cells may be critical to their proliferative potential. Phosphatidic acid phosphohydrolase (PAP), which dephosphorylates PA to generate diacylglycerol (DAG), may therefore have a pivotal role in regulating cell function. Distinct PAP isoforms have been described in a number of cell types (Jamal *et al.*, 1991; Jamdar & Cao 1994). We have previously demonstrated the presence of a PAP-2 type activity in membrane fractions of GPASM cells (Tolan & Pyne 1995). The present work investigates the species specificity of PAP-2 type activity in membrane fractions of GPASM cells and examines the regulation of this activity.

Primary cultures of GPASM cells were prepared from a guinea-pig tracheal smooth muscle strip. Cells were grown to confluency in DMEM supplemented with 10% FCS/10% DHS and utilised for experiments between 16-21 days after initial preparation. Membranes were obtained by centrifugation (48 000 x g for 20min). PAP activity was measured by assaying the release of [^{32}P]Pi from [^{32}P]-PA. The Assay method was validated by thin layer chromatography analysis of reaction products. Assays were performed at 30°C for 5min using 150μM PA (~6500dpm/nmol). Triton X -100 was included in the assay at a fixed ratio of PA : Triton X -100 (1:10).

A PAP activity has been detected in membrane fractions of GPASM cells. This activity has been found to be Mg^{2+} independent and insensitive to inhibition by NEM, suggesting that it is a PAP -2 type activity. Sphingosine inhibited this activity in a concentration dependent manner. PAP activity exhibited no preference for PA species containing long or short acyl chains (dioleoylPA C18:1,18:1 100% activity, dioctanoylPA C8:0:8:0 84.5±29% activity). However PAP activity was least active against PA species containing stearoyl/arachidonyl acyl chains (C18:0,20:4 35.4±7.1% activity). Furthermore, pre-incubation of the membranes with a non-hydrolysable analogue of GTP (GppNHp) had no effect on PAP activity. All values represent mean±sd for three independent experiments.

These results suggest that the PAP-2 type activity in GPASM cells is more active against PA species derived from phospholipids other than the phosphoinositols which are rich in stearoyl/arachidonyl acyl chains. Furthermore, there appears to be no direct regulation of membrane PAP activity by G-proteins.

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459P COMPUTER-ASSISTED COURSEWARE IN DRUG METABOLISM

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The study of drug metabolism is an integral part of Pharmacology courses but often suffers from being heavily chemically-orientated with many drug structures and chemical reactions to master. A lecture-based course in drug metabolism that keeps the students' attention is somewhat difficult to devise as it can involve showing many different reaction sequences and mechanisms of reaction without the ability for animation. On this basis, we have designed and implemented a computer-based courseware package in drug metabolism using animated reaction sequences.

The courseware is designed as an introduction to drug metabolism for science, dental, medical and pharmacy students. No prior knowledge of drug metabolism is assumed although a grounding in chemistry and chemical reactions is useful to make best use of the courseware. The aim of the courseware is to give students an understanding of the basic principles of drug metabolism including routes of metabolism. The courseware runs on a PC with 386 processor running at a minimum of 25MHz with VGA 16-colour graphics capability. Windows 3.1 is also required. The courseware comes as a self-loading archive file.

The courseware covers where drug metabolism occurs, why it is important and the major routes of phase 1 and phase 2 drug metabolism (including the enzymes and cofactors involved). The synthesis of cofactors involved in phase 2 metabolism is also covered as is the cytochrome P450 catalytic cycle including the electron transport chain, the nomenclature of cytochromes P450 and the further metabolism of glutathione conjugates. There are short quizzes after each section and a longer integrative quiz, based on aspirin metabolism, at the end of the courseware. The quizzes can be by-passed if the student is quickly revising. The direct route through the courseware can be completed in about 45 minutes but can be extended to 9 hours if all information is used. The courseware can be seen as complementary to the textbook, "Introduction to Drug Metabolism" (Gibson and Skett, 1995).

The courseware has been evaluated and checked in a number of Departments over the past year and is now available in its final version.

This courseware has been developed by the Pharma-CAL-ogy Consortium under TLTP funding from the HEFCE.

Gibson,G.G. and Skett,P. (1995). "Introduction to Drug Metabolism", Chapman & Hall, London, pp 1-266.

460P THE USE OF COMPUTER SIMULATIONS TO ACCOMPANY THE LABORATORY TEACHING OF PHARMACOLOGICAL PRINCIPLES

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In addition to developing technical skills, practical classes are useful for demonstrating a range of pharmacological principles. However, as aids for teaching basic principles, such classes have some limitations. Whole animal experiments require ethical justification while electrophysiological experiments are technically complex and require expensive equipment. Experiments using isolated tissues are more readily accessible to students but occasionally these experiments either do not work or give unexpected and unexplained results. Finally, time is often a limiting factor. To circumvent some of these problems, we have developed a suite of computer based practical simulations that can be used in conjunction with normal laboratory classes. The simulations are not designed to replace either the laboratories themselves or the need for academic guidance but serve as a means to extend and reinforce the learning processes associated with the classes. In addition to formal time-tabled sessions, all simulations are available to the students through the relevant course and can be used for revision work in their own time.

The simulations have been written using LabWindows or Visual Basic. Each is based on a classical animal experiment and is a representation of the experiment itself rather than a tutorial describing the experiment. LabWindows provides an excellent programming environment for the creation of on-screen likenesses of pen recorders and oscilloscopes and is therefore particularly useful for this style of presentation. All simulations use algorithms to model drug-response relationships rather than the "library-of-responses" approach and while this makes them harder to design, they are more flexible in their use.

At present, six simulations are used. In the *Anaethetised Cat Simulation*, students can determine the effects of a range of drugs on blood pressure, heart rate, skeletal twitch tension and contractions of the nictitating membrane. In the *Pithed Rat Simulation*, the effects of catecholamines and sympathetic nerve stimulation on blood pressure can be investigated. These "*in vivo*" simulations can be used to determine responses to a number of "unknowns" used in the associated laboratory classes. The *Guinea-pig Isolated Ileum Simulation* allows the calculation of antagonist pA_2 values. The remaining three simulations explore neuromuscular transmission at a number of different levels. The *Rat Isolated Hemi-Diaphragm Simulation* looks at drug effects on twitch tension, the *Electrophysiological Simulation* allows the exploration of motor endplate electrical activity and the *Ion Channel Simulation* determines the effects of agonists and antagonists (both competitive and non-competitive) on a patch-clamp record of ion channel activity.

The simulations are currently used in three out of four years of our degree courses and some have now been in use for four years. Feedback suggests that they are popular with the students both as complements to the practical classes and as a means to the understanding of pharmacological principles. Source codes for the simulations are available free of charge using anonymous FTP from ppserver.dpp.strath.ac.uk (130.159.32.4).

461P REPORT OF A WORKSHOP ON PROBLEM-BASED LEARNING AS APPLIED TO PHARMACOLOGY COURSES

This workshop was organized by the *Pharmacology Higher Education Network* as part of the meeting of the British Pharmacological Society held at Strathclyde University. Among the aims of the Network are to encourage academic staff to use active learning methods which will develop personal skills in our graduates and enhance their learning and employability.

The Chairman, Dr M Hollingsworth, opened the workshop by welcoming the 50 participants. He referred to the first workshop held in April 1995 on "What's required in a pharmacology graduate?" This workshop addressed the skills, knowledge and competencies required by pharmaceutical and non-pharmaceutical employers, for which there was considerable uniformity of needs (*Br. J. Pharmacol.*, **115**, 161-168P).

The current workshop built on that base by addressing the ways in which problem-based learning methods could encourage the development of these characteristics. Many universities are re-examining or instituting major changes in course design. This workshop, therefore, dealt with the educational basis of problem-based learning and the practicalities of incorporating such methods in new courses.

Further details of the Pharmacology Higher Education Network can be obtained from the Convenor of the Network, Dr Ian Hughes, Department of Pharmacology, University of Leeds, Leeds LS2 9JT; tel 01132-334313; fax 01132-334331; e-mail i.e.hughes@leeds.ac.uk