343P CHARACTERISATION OF CYSTINE ACCUMULATION INTO RAT CEREBELLAR SLICES AND INHIBITORY CHARACTERISTICS of 2-S-L-CYSTEINYL PROPIONIC ACID

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Oral administration of L-2-chloropropionic acid (L-CPA; 750 mg/kg) to Alderley Park (AP) rats produces selective cerebellar granular cell necrosis within 48 hours following administration (Widdowson et al., 1995). This treatment also depletes cerebellar glutathione (GSH) in a time dependent manner with maximal depletion occurring 24 h after dosing (Wyatt et al., 1995), prior to granule cell necrosis. The depletion of GSH was not due to conjugation with L-CPA or as a result of oxidative stress (Wyatt et al., 1995). Preliminary studies had indicated that reduced L-cystine transport into the cerebellum, might be involved in the GSH depletion produced by L-CPA, since 100 µM L-2-cysteinyl propionic acid (2-CP) the cysteine conjugate of L-CPA, inhibited 10 µM [^{14}C]cystine accumulation into cerebellar slices by 50%. L-cysteine is one of the amino acids required for GSH synthesis (however, in-vitro, cysteine is rapidly converted to cystine which is transported into the brain as cystine and then rapidly reduced to L-cysteine once inside cells). We examined the kinetics of transport of cystine into the cerebellum and the effect of 2-CP on this process to see whether this may account for the decrease in cerebellar GSH seen after L-CPA treatment. Male AP rats (200-220 g) were killed by halothane overdose. Cerebellar slices (0.5 mm thick) were cut using a McIlwain tissue chopper and incubated at 37 °C under O_2 in Krebs buffer (pH 7.4) containing 5, 10, 20, 50, 100 and 200 µM [^{14}C]cystine. The accumulation of radiolabelled [^{14}C]cystine was measured at 0, 30, 60, and 120 min with three separate analyses performed using cerebellar slices from different animals. The rate of accumulation of [^{14}C]cystine was expressed as nmol/g wet weight/h and determined for each concentration using a linear regression fit. The accumulation of cystine into cerebellar slices was found to obey saturation kinetics with an apparent Km of 77 µM and a Vmax of 450 nmol/g wet weight/h using a linear regression fit to the data (correlation coefficient = 0.99). To characterise the inhibition of cystine accumulation further by 2-CP, cerebellar slices were incubated in 5, 10, 20, 50 and 100 µM [^{14}C]cystine with and without 100 µM 2-CP, for 2 h at 37 °C under O_2 with 3 separate determinations per concentration. The rate of accumulation was determined by subtracting the zero time point intercept, obtained from the studies used to derive the saturation kinetic constants, from the concentration of cystine found at 2h. Using a Hane plot and linear regression analysis, the inhibition observed was non-competitive with correlation coefficients of 0.93 and 0.97 for control and 100 µM respectively giving a Ki for 2-CP of approximately 60 µM. The addition of 1 mM L-glutamate to cerebellar slices gave only diffusion rates for [^{14}C]cystine uptake. The lack of active transport of [^{14}C]cystine into the slices was due to L-glutamate-induced cytotoxicity. Cytotoxicity was monitored by incubating cerebellar slices in [1-^{14}C]glucose and measuring the evolution of [^{14}C]CO_2 over 2 h. 1 mM L-glutamate reduced [^{14}C]CO_2 production by 18% ($P>0.01$) for 4 observations (Student's t-test) demonstrating a decreased cellular metabolism. The non-competitive inhibition of [^{14}C]cystine uptake into cerebellar slices by the metabolite of L-CPA, 2-CP suggests that the L-CPA-induced reductions in GSH concentrations in the cerebellum *in vivo* could be due to reduced GSH synthesis as a result of a reduction in substrate supply, notably cysteine.

Widdowson, P.S., Simpson, M.G., Wyatt, I., Lock, E.A. (1995) *Peptides* 16: 897-902.
Wyatt, I., Widdowson, P.S., Gyte, A., Moore, R.B., & Lock, E.A. (1995) *Br. J. Pharmacol.* 116: 102P.

344P A COMPUTER SIMULATION TO DEMONSTRATE THE EFFECTS OF PHARMACOLOGICAL AGENTS OR PROCEDURES ON BLOOD PRESSURE AND HEART RATE OF THE ANAESTHETIZED RAT *IN VIVO*

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In recent years a large number of computer programs which simulate undergraduate pharmacological experiments or preparations have been demonstrated to the Society. These have a number of potential uses. For example, they may be used: to better prepare students who will the perform the experiment(s) at a later date; to debrief students after they have performed the experiment(s); as a fallback to provide data for students whose experiments were unsuccessful; as an alternative to real experiments if equipment and/or expertise is not available in a particular department. This program simulates a range of experiments which may be performed on the anaesthetized rat (*in vivo*) to investigate the action of a number of pharmacological agents or procedures on blood pressure and heart rate. It is suitable for undergraduates from science, medical and a range of biomedical courses which include pharmacology modules.

The program was developed using Multimedia Toolbook® (Asymetrix) to run on IBM PC compatibles (minimum delivery platform: 386 SX, 20 MHz PC running Windows™ version 3.1 (Microsoft), a sixteen colour VGA monitor and a mouse). The main menu allows students to access sections covering different aspects of the laboratory class. **Introduction** covers Home Office Licence requirements; **Preparation** covers anaesthesia and anaesthetization, cannulation of trachea, jugular vein and carotid artery; **Apparatus** covers the equipment used to maintain body temperature, record blood pressure and heart rate; **Measurements** describes how to take measurements from the simulated chart recorder display and how to calculate mean BP and pulse pressure. Each of these sections combines text, high quality colour graphics, and animation with interactive questions designed to reinforce learning. **Experiments** is a large section providing typical data for 16 different experiments.These include actions of: catecholamines (noradrenaline (NA), adrenaline, isoprenaline); pressor agents (adrenaline, vasopressin, phenylephrine); acetylcholine (acetylcholine (high and low doses), atropine); ganglion stimulants (DMPP in normal and atropinised animal); uptake$_1$-blockers (cocaine on NA, tyramine, phenylephrine); alpha-blockers (prazosin on NA, vasopressin and phenylephrine); beta-blockers (propranolol on isoprenaline, adrenaline); adrenaline reversal (NA, NA+prazosin, NA+prazosin+ propranolol); guanethidine (on NA, vasopressin, sympathetic nerve stimulation (SNS)); SNS (effects of hexamethonium, prazosin, propranolol and guanethidine on responses to NA and SNS); depressor drugs (histamine, acetylcholine, isoprenaline, hexamethonium) ; ganglion blockade (hexamethonium on responses to NA, vasopressin); quantitative effects of alpha-blockade (several doses of adrenaline with/without prazosin); quantitative effects of beta-blockade (several doses of adrenaline with/without propranolol); reserpine (NA, tyramine, and SNS in normal and reserpine-treated rats); pithing (NA, tyramine and SNS in normal and pithed rats.

Students are expected to record and tabulate data from the screen display and to then complete student assignments. Two different assignments are offered for each experiment: (i) a series of MCQ questions, with feedback, to assess accuracy of data collection and data interpretation; (ii) a student task (typical of a traditional lab-class report) to be completed in their own time. Tutors may direct students to complete either or both of these tasks or design their own assessments. In addition there is a section containing a selection of MCQ's with feedback covering cardiovascular pharmacology which students can use for revision.

345P A COMPUTER-BASED INTERACTIVE TUTORIAL PROGRAM TO TEACH THE PHYSIOLOGY OF THE CIRCULATORY VESSELS, BLOOD FLOW AND BLOOD PRESSURE TO UNDERGRADUATE STUDENTS

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Computer-assisted learning (CAL) has a number of advantages: it promotes active learning; gives students control over when and where they learn and the pace of their learning; promotes better understanding by using features which enhance the quality of presentation of material and student interaction; potentially saves staff time. At a recent meeting of the Society we demonstrated an interactive tutorial program designed to teach the physiology of the heart (Dewhurst & Dawson, 1995). Here we demonstrate a similar program which covers the physiology of the blood vessels, blood flow and regulation of blood pressure. It is suitable for first year, foundation level undergraduates from a range of biological science, medical and health-related courses and may be used for both primary learning, revision and as a remedial teaching resource.

The program was developed using Authorware Professional® (Macromedia Inc.) to run on IBM PC compatibles (minimum delivery platform: 386 SX, 20 MHz PC running Windows™ (Microsoft), a sixteen colour VGA monitor and a mouse).

The main menu has three options: which may be accessed in any order: **Aims and Objectives**; **Introduction** (covers structure and function of the circulatory system); and **The Circulatory vessels.** This latter section has a submenu: *structure and function* (generalised structure of the blood vessel wall, structure and function of arteries, arterioles, capillaries, venous vessels); *blood flow* (factors affecting blood flow, pressure and resistance); *peripheral resistance* (resistance vessels and their control); *blood pressure* (measurement of blood pressure, regulation of blood pressure including autonomic reflexes, hormones, intrinsic regulation, long-term control by the kidneys).

High quality colour graphics are used extensively throughout the program and features such as animation and a Hypertext facility are used to enhance student learning. The program is highly interactive and uses several features to promote this. For example questions, calculations and simulations are integrated into the program to reinforce learning and in some instances to further develop certain areas which students often have difficulty with. A variety of questions types are used including: labelling diagrams by "dragging" labels from a list and "dropping" them into the box corresponding to the correct position on the diagram; multiple choice with feedback; selecting correct phrases from a list to complete a statement, and true/false questions with feedback.

The learning package is intended for independent study and could be used as an alternative to resource-intensive, staff-led tutorials or lectures (the material covers approximately four one-hour lectures to first year students). It is estimated that it would occupy students for 3-5 hours of fairly intensive study and is suitable for primary learning or revision. The question-answer sections may also be useful for self-assessment.

Dewhurst D.G. & Dawson, O.M. (1995) Brit. J. Pharmac. Proc. suppl. (in press).