140P THE EFFECT OF HO-1 INDUCTION ON THE POST-ISCHAEMIC RECOVERY OF THE ISOLATED RAT HEART

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HO-1, which can be induced by hemin (*Maines, M.D.,1997),* is reported to be involved in a number of situations where oxidative stress occurs, and has been demonstrated to be up-regulated upon cardiac reperfusion (*Maulik et al.,1996*). HO-1 is involved in the breakdown of haem resulting in the formation of bilirubin, which has antioxidant properties and CO, which is a potent vasodilator. Bilirubin has been suggested as the agent responsible for the protective role of HO-1 in ischaemia-reperfusion injury (*Clark et al.,2000).* In the present study, we have compared the effect of hemin pre-treatment on the recovery of cardiac function in the isolated rat heart under conditions of ischaemia-reperfusion using a constant flow perfusion sytem. Tin protoporphyrin, a known inhibitor of HO-1 (*Maines, MD 1981*) was also used to investigate the effect of HO-1 inhibition in this model.

Male Wistar rats (270-320g) were pre-treated with 75μmol/kg Hemin (24 hours) or 40μmol/kg tin protoporphyrin (1 hour) intraperitoneally, and anaesthetised with Euthatal (120mg/kg). Hearts were removed and perfused using the Langendorff technique with Krebs-Henseleit solution (118mM NaCl, 1.25mM $CaCl_2$, 1.2mM KH_2PO4, 2.8mM KCl, 1.2mM $MgSO_4$, 25mM $NaHCO_3$, 11.1mM glucose) gassed with 95% O_2/5% CO_2 at a constant flow rate of 10ml/min. After a period of 30 minutes stabilisation, a 20 minute period of zero flow global ischaemia was introduced; this was followed by 30 minutes of reperfusion. Measurements recorded included left ventricular developed tension (LVDT), heart rate and coronary perfusion pressure (CPP). Tissue was then prepared for immunoblot analysis, which was carried out using a mouse anti-rat HO-1 antibody. Data are presented as mean±SEM; significant differences were calculated using ANOVA.

Immunoblot analysis determined that the dose of hemin used increased the expression of HO-1. Under pre-ischaemic conditions, a significant decrease in coronary perfusion pressure was observed in the hemin-treated rats compared to control (116±9 mmHg with hemin compared to 141±3 mmHg for control; $p<0.05$, n=5), indicating a vasodilatory component. This effect was abrogated using the inhibitor tin protoporphyrin, where hemin and inhibitor increased the CPP to 131 ± 10mmHg. After ischaemic injury, hemin pre-treatment significantly increased recovery of contractility (recovery to 45±8% of initial levels in hemin-treated rats, compared to 14 ± 4% for control, where $p<0.05$ n=5) and a significant reduction in CPP compared to control from 2-30 minutes post-ischaemia ($p<0.05$, n=5). The addition of tin protoporphyrin partially reduced the recovery of contractility to 27±13% (compared to 7±3% in rats treated with inhibitor alone), and attenuated the post-ischaemic effect of hemin on CPP.

These data suggest that hemin pre-treatment results in an increased recovery from ischaemic injury. Further data obtained using a HO-1 inhibitor, and also immunoblot analysis, indicate that these effects may be due to the induction of HO-1 by hemin. The effects of hemin on CPP suggest a vasodilatory component, possibly via CO release.

Clark et al.,2000, Am.J. Physiol., 278, H643-H651
Maines, MD. 1981, Biochim. Biophys. Acta 673, 339-350
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Maulik et al.,1996, J.Mol.Cell Cardiol. 28, 1261-1270

141P A HIGHLY INTERACTIVE COMPUTER ASSISTED LEARNING (CAL) PROGRAM TO TEACH BETTER EXPERIMENTAL DESIGN

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In excess of 50 million animals are used in biomedical research in the world each year (Festing, 1996). For both ethical and economic reasons it is important that research scientists use experimental animals efficiently and in the minimum numbers consistent with achieving the scientific objectives of the study. More effective experimental design could help to achieve a significant reduction in the number of animals used and, by improving the repeatability of animal experiments, could make alternative methods easier to validate. Here we describe a CAL program designed to teach scientists how to estimate the number of animals needed, the importance of uniformity, how to deal with variability and how to increase efficiency and therefore cost effectiveness of their experiments. It is aimed at all research scientists using experimental animals, but the principles of experimental design are applicable to most areas of biological and medical research. Briefly the content covers:

Introduction - highlights poor design in a significant proportion of animal experiments and consequent unnecessary use of animals. The section engages the user with data from a simple experiment to highlight design flaws.

Choice of Animal Model - explores the use different strains (inbred and outbred stock) and covers the various types of animal model (predictive, explanatory, exploratory).

The Experimental Unit - the importance and critical nature are explained by using interactive examples.

Eliminating Bias – uses interactive examples to explain the techniques which may be employed to remove systematic differences between treatment groups and reduce bias.

Applying Valid Statistics - covers the application of valid statistical tests to your data, the use of statistical tests to compare two groups, parametric versus non-parametric tests, assumptions underlying parametric tests, and the way in which they can be examined. Explores the definition of hypotheses, choices of statistical tests, interpretation of P. Introduction to the analysis of variance (ANOVA).

Improving Precision - making experiments more precise so that we can detect treatment differences. Ways of achieving this - ensuring uniformity, use of randomised blocking as a way of minimising heterogeneity, using power analysis and the resource equation method.

Increasing the Range of Applicability - using resources effectively to enable interpretation of findings over a wider range e.g. different treatments, different strains, sexes, sizes. Use of multi-factorial design.

Planning and Organising - key issues in designing and analysing effective (simple) experiments.

Self-Assessment Activity - series of case studies and true/false questions with feedback to self-assess your understanding.

Software Tools & References – other information resources.

The program requires a PC with a minimum specification of 166 MHz Intel Pentium II processor, Windows 95/98/NT4, 32 Mb RAM, 16 bit colour graphics.

Festing (1996) *Statistics in Toxicology* (Morgan B. J. T., ed), pp. 3-11. Clarendon Press, Oxford, ISBN 0 19 8523297.