D1

Automatic determination of ligand purity and apparent equilibrium constant in divalent cation buffer solutions and apparent equilibrium constant in physiological solutions

J.W. Kay[1], R. Stevens[2], J.A. McGuigan[2] and H.Y. Elder[2]

[1]Statistics, University of Glasgow, Glasgow, UK and [2]I B L S, University of Glasgow, Glasgow, UK

Various proposed methods for the determination of ligand purity and apparent equilibrium constant (K_{app}) in divalent cation buffer solutions are discussed by McGuigan *et al.* (2004) who show that the obstacles present in the other existing methods are overcome by the method of Luethi *et al.* (1997). However, this method involves laborious, iterative calculations and therefore it is not generally used.In the method of Luethi *et al.* (1997) the problem is to determine three parameters: ligand purity, K_{app} and the nonlinearity parameter in the Nicolsky-Eisenmann equation. This problem can be re-formulated using a constrained nonlinear least squares approach in which a single objective function of all three parameters is defined and minimised subject to the constraint that the ligand concentration is no greater than its nominal value. The Nicolsky-Eisenman equation relates the measured potentials in the buffer solutions to the values of the divalent cation. From Luethi *et al.* (1997), the concentration of the divalent cation may be written as a function of the total divalent cation concentrations together with the ligand purity and K_{app}. By substituting this equation for the divalent cation into the Nicolsky-Eisenman equation a single nonlinear regression model is obtained which directly links the measured potentials with the corresponding values of total concentrations of the divalent cation. The associated residual sum-of-squares function is then a single objective function which may be directly minimised with respect to all three unknown parameters, subject to the constraint on the ligand concentration. The method of Luethi *et al.* (1997) cannot be used at values of K_{app} greater than 0.1 mmol/l. However, if the concentration of the organic anion is known, the method can be easily modified to determine K_{app}. The binding of Mg^{2+} to malate illustrates both aspects.This automated approach has been implemented in an Excel spreadsheet using the freely-available Solver Add-In and it is driven by macros which have been written in Visual Basic. The programme is easy to use and, once the initial data have been entered and options selected, the solution is produced literally at the touch of a button. Given familiarisation, this programme makes the method proposed by Luethi *et al.* (1997) widely available for fast computation of the ligand purity and K_{app} of divalent cation buffer solutions and of K_{app} for divalent cations binding to organic anions of physiological importance (e.g. malate, citrate and aspartate). The programme which is demonstrated at this meeting is available for PC use from Jim Kay (jim@stats.gla.ac.uk).

Luethi D et al. (1997) Exp. Physiol. 82 53-467

McGuigan JAS et al. (2004) This Meeting

D2

Determination of mechanical energy thresholds of *commotio* vs. *contusio cordis* in Guinea pig isolated heart

I.A. MacLeod[1], P.J. Cooper[1], S.T. Schaaf[1], A. Epstein[2], C. Boulin[2] and P. Kohl[1]

[1]University Lab of Physiology, Oxford, UK and [2]EMBL, Heidelberg, Germany

Commotio cordis [shaking of the heart] refers to mechanically induced disturbances in heart rhythm, usually brought about by impacts to the pre-cordial chest. In contrast to *contusio cordis* [bruising of the heart], *commotio* is not accompanied by structural damage to the chest and its organs (in particular the heart) that could explain the observed electrophysiological effects. Case registries, whole animal experiments, and theoretical considerations have identified a number of risk factors and probable mechanisms (reviewed in [1]). Currently, there is no isolated heart model of the condition, which makes it difficult to assess (sub-)cellular mechanisms. Even the actual energy threshold at which *commotio* turns into *contusio cordis* (i.e. when tissue damage occurs) is, as yet, unknown. A previous report suggested that impacts of up to 200 mJ cause only superficial tissue damage in rabbit isolated heart [2]. This study is designed to assess that suggestion.Mechanical impacts were performed using a custom-built device [3], comprising a low-friction sliding mechanism that carries a probe of variable weight and impact surface area (Fig. 1). The probe is released from a user-defined height (triggered manually or by computer), and its position immediately prior to and during impact is measured using an optical grid system (resolution < 0.2 mm), interfaced with a BioPac MP150 (200 kHz per channel), and AcqKnowledge software. Upon complete deceleration, a hardware-controlled retractor arm swiftly removes the probe from the tissue to prevent secondary interaction. Based on known probe mass and impact area, and on measured pre-impact speed and deceleration characteristics, we obtain precise data on parameters such as force, pressure, deformation and work.Impacts were targeted at the left-ventricular free wall of isolated, Langendorff-perfused hearts from Guinea pigs (killed by cervical dislocation; all investigations conform with UK Home Office Regulations). Hearts were positioned in a cradle underneath the impactor and perfused with either Krebs-Henseleit (KH) solution (in mM: NaCl 118, $CaCl_2$ 1.8, KCl 5.4, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, Glucose 10; carbogen bubbled to pH 7.4), cardioplegic KH ($[K^+]$ raised to 20 mM), or subjected to contracture (KH equivalent where Na^+ was replaced by Li^+); all at 37°C. Up to three impacts were performed on each heart, and their position was documented by still and video photography.Tissue integrity was judged on site by photometric creatine kinase (CK) assay (340 nm absorbance, assay resolution 4.18 IU/L), and verified in subsequent histological studies [4]. For CK assays, coronary outflow was collected before impact (control), 3-4 times during the first minute after impact, and then at 2, 3, 5, 10, 20, and 30 min. Contrary to prior work [2], we found that samples should not be frozen but stored at 4°C, as this preserves >98% enzymatic activity for up to 24 h, whereas immersion in liquid nitrogen instantaneously reduces CK activity to <30% of the pre-freezing value and exposure to dry ice causes a reduction to <50%.Maximum pre-impact kinetic energies that did not induce tissue damage, identifiable by either CK assay or light

microscopy, were 2.0-2.5 mJ for cardioplegically arrested hearts, and 5-10 mJ for contractured preparations. Tissue damage correlated well with the deceleration pathway length, which was shorter in contractured hearts. Since force (and, hence, peak pressure under the probe) is inversely related to deceleration pathway length, this suggests that the critical parameter in introducing tissue damage may, in the given experimental conditions, not be the compression of the tissue under the probe, but distension of neighbouring tissue.In the context of *commotio cordis*, the heart is particularly prone to developing fatal arrhythmias if impacts occur during the early T-wave. This coincides with ventricular contraction, which, according to our findings, would extend the relevant (sub-contusional) pre-impact energy range. Subsequent investigations will assess whether pre-impact speed is an independent determinant of tissue damage, and then move on to electrophysiologically active beating heart preparations.

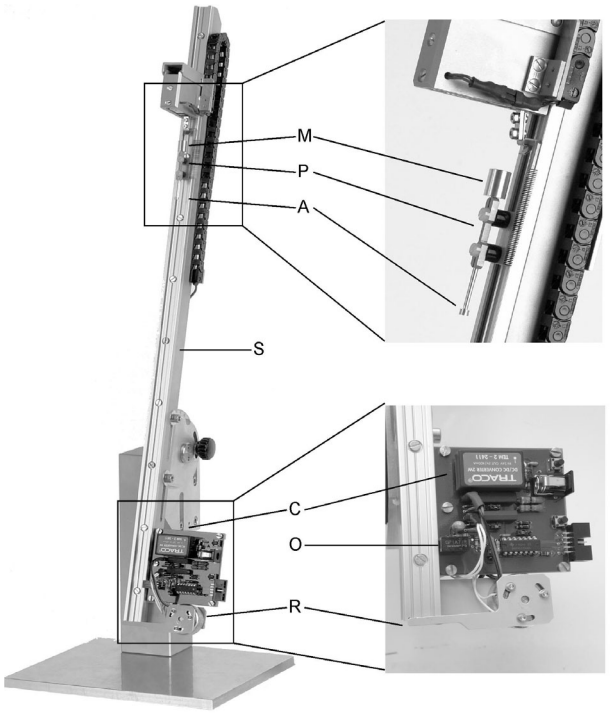

Figure 1: Photograph of the Soft Tissue Impact Characterisation Kit (STICK) Low friction slide (S), probe (P) with variable impact area (A) and mass (M), control circuitry (C) with optical sensor (O) and retractor arm (R)

[1] Kohl P *et al.* (2001) *Cardiovasc Res* **50**:280-289.

[2] Robert E *et al.* (2000) *Anesthesiology* **92**:1132-1143.

[3] Boulin C *et al.* (2004) *Biophys J* (abstract of the Biophysicial Society Meeting, February 2004).

[4] Schaaf STM *et al.* (2004) J *Physiol* (abstract of this Physiological Society Meeting, March 2004).

Supported by the British Heart Foundation. PK is a Royal Society Research Fellow.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.