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Calcium and magnesium buffer solutions: the need for standardisation

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To measure intracellular Ca^{2+} concentrations and the binding of Ca^{2+} and Mg^{2+} to physiological compounds one must manufacture respectively nmolar and µmolar buffers. To calculate the free ionic concentration the apparent equilibrium constant (K_{app}) in the buffer solution has either to be measured or calculated with appropriate temperature, ionic strength and pH. Due to the binding of water, the ligand concentration must also be determined. The principal experimental methods are based on partition methods, macroelectrodes, calcium indicators and pH titration. Only macroelectrodes and pH titration measure both purity and K_{app}. The pH method of Moisescu & Pusch (1975) accurately determines ligand purity but for K_{app} determinations assumes incorrectly that 2 H^+ ions are released for 1 Ca^{2+} ion bound. It is only an approximation. The Bers (1982) method using Ca^{2+} macroelectrodes cannot be used with Mg^{2+} macroelectrodes (Luethi *et al.* (1997). Unless the Ca^{2+} macroelectrode is linear, the method overestimates both purity and K_{app} and also has a subjective element. The improved iterative method (Oiki *et al.* 1994) cannot be used with Mg^{2+} macroelectrodes. The most general method to measure both K_{app} and ligand purity (Luethi *et al.* 1997) is also based on macrolelectrodes, does not depend on the linearity of the macroelectrode and is not subjective but it is iterative, tedious and time consuming. These problems have now been overcome with a MS Excel program (Kay *et al.* this meeting, C78). An additional problem with such buffer solutions is the precision of the measurement. However, there are no defined limits of allowable coefficient of variation (CV). If ligand purity is known then K_{app} can be calculated from internet programs (Maxchelator, BAD and Chelator) or calculated directly from the constants in Martell & Smith (1974). From the various programs only Chelator is similar to the experimentally estimated calcium concentration in Ca^{2+} buffer solutions. In conclusion, there is no defined method to estimate ligand purity and K_{app}, no upper limit to CV and calculated concentrations can vary by a factor of 2 for Ca^{2+} or between 3 and 4.5 for Mg^{2+} (Luethi *et al.* 1997). International standardisation of Ca^{2+} and Mg^{2+} buffer solutions is now needed.

Bers DM (1982) *Amer. J. Physiol.* **242**, C404-C408

Luethi D *et al.* (1997) *Exp. Physiol.* **82** 453-467

Martell AE & Smith RM (1974) *Critical Stability Constants, volume 1, Amino Acids* Plenum Press: New York 469 pp

Moisescu DG & Pusch H.(1975) *Pflugers Archiv.* **355** R122

Oiki S *et al.* (1994) *Cell Calcium* **15** 199-208

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The AMPK-PKB switch: a possible mediator of specific phenotype or growth adaptations in response to endurance and resistance training-like electrical stimulation

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A simple comparison between a marathon runner and a weight lifter shows that an increase in contractile activity can trigger very different adaptations in skeletal muscle. We investigated whether a selective activation of AMP kinase (AMPK) or protein kinase B (PKB; Akt) and its downstream translation factor p70 S6 kinase (p70 S6k) could be responsible for phenotypic or growth adaptations to different forms of exercise.Male rats were humanely killed and extensor digitorum longus (EDL) or soleus muscles were placed in oxygenated Krebs-Henseleit buffer. Muscles were electrically stimulated either continuously at 10 Hz for 3 h (endurance training-like stimulation; ETS) or for 10 sets of 6 repetitions of 100 Hz bursts, each of 3 s duration, with 10 s recovery between repetitions and 1 minute of recovery between sets (resistance training-like stimulation; RTS). Repeated RTS was previously shown to induce hypertrophy in vivo whereas ETS resulted in endurance training-like adaptations (Nader & Esser, 2001). The phosphorylation of AMP kinase (AMPK) at thr172, of protein kinase B (PKB/Akt) at thr308, and of the translation regulator p70 S6k at thr389 were estimated by densitometry of Western blots on protein extracts and calculated relative to control. The selective activation of AMPK by ETS is consistent with the AMPK-dependent activation of mitochondrial biogenesis and other adaptations commonly observed in response to endurance training. In contrast, the selective activation of PKB and p70 S6k by RTS is consistent with the increase in protein synthesis that is observed after resistance training. To conclude, the AMPK-PKB switch is likely to be an important switch that regulates specific adaptation to endurance and resistance training. The results also indicate that stimulation of isolated skeletal muscle is a suitable model for investigating signalling responses to exercise.

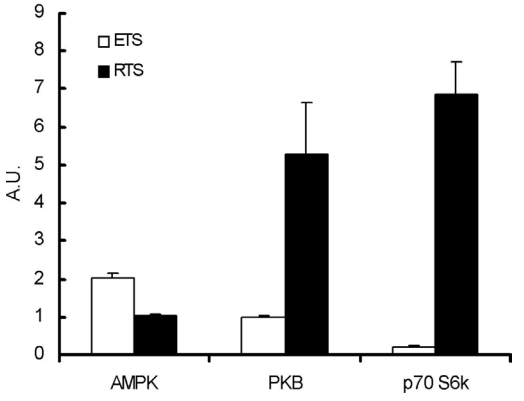

Figure 1 (mean+/-SEM; n=8 for each bar, 4 EDL and 4 soleus pooled) indicates that ETS but not RTS increased AMPK

phosphorylation. In contrast, RTS but not ETS activated PKB-p70 S6k signalling. The increase in AMPK phosphorylation after ETS, the increases in PKB and p70 S6k phosphorylation after RTS, and the decrease in p70 S6k phosphorylation after ETS were all significantly different from control (ANOVA; $p<0.05$).

Nader GA & Esser KA (2001) 90, 1936-1942.

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