## Glycogen content and contractile responsiveness to T-system depolarization in skinned muscle fibres of the rat

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It has been reported<sup>1</sup> that when toad mechanically skinned fibres are incubated in aqueous solutions mimicking the myoplasmic environment at rest, they retain a large proportion of glycogen and a  $Ca^{2+}$ -sensitive glycogenolytic system that is activated during T-system depolarization-induced isometric contraction. Furthermore, it was shown that in these fibres, initial fibre glycogen content and fibre responsiveness to T-system depolarization are positively correlated under conditions of high [ATP] and [phosphocreatine]<sup>1</sup>. This latter finding strongly suggests a non-metabolic role of glycogen in vertebrate skeletal muscle contractility. In this study we aimed to examine the applicability of the toad protocol for studying, at the cellular level, glycogen metabolism and its role in muscle contractility in rat skeletal muscle.

Glycogen content (determined microfluorometrically)<sup>2</sup>, fibre response capacity to T-system depolarization<sup>1</sup> and the relationship between these two parameters were examined in mechanically skinned fibres from rat EDL muscle in the presence of 8mM ATP and 10mM phosphocreatine at 20-25°C. All animals were killed by halothane overdose in accordance with Victoria University AEEC procedures.

Total glycogen content in EDL fibres was  $58.1 \pm 4.2$  mmol glucosyl units/l fibre (n=53), with a large proportion being retained in skinned fibres (SFGlyc) after 2min (73.1 ± 2.8%) and 30min (64 ± 2.3%) exposure to an aqueous relaxing solution. The proportion of SFGlyc was 72% lower after 30min incubation in a high (30 m M) Ca<sup>2+</sup> solution, suggesting that rat skinned fibres retain a Ca<sup>2+</sup>-sensitive glycogenolytic complex. T–system depolarization-induced Ca<sup>2+</sup>-release was not associated with any detectable fibre glycogen loss even though fibre excitability diminished. This indicates that under our conditions, other factors, unrelated to glycogen depletion, may have limited the capacity of rat skinned fibres to respond to T-system depolarization.

It is concluded that (i) rat mechanically skinned fibre preparations are well suited for studying glycogenolysis at a cellular level and (ii) the experimental conditions/protocol used for toad<sup>1</sup> may have to be modified for further examination of a potential non-metabolic role of glycogen in mammalian skeletal muscle contractility.

(1) Stephenson et al. (1999) J Physiol 519:177-187
(2) Nguyen et al. (1998) J Muscle Res Cell Motil 19:631-638