## Functional and electrophoretic identification of two Troponin C isoforms in toad skeletal muscle fibres

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Activation of contraction in striated muscle of vertebrates is regulated by the binding of $Ca^{2+}$ to the myofibrillar protein Troponin C (TnC). In mammals, TnC is known to exist as two isoforms, one found in fast-twitch skeletal muscle (TnC-f), the other found in both slow-twitch skeletal and in cardiac muscle (TnC-s/c) (Gomes *et al*., 2002). These isoforms confer to fibres in which they are expressed different contractile activation characteristics with respect to $Ca^{2+}$ and $Sr^{2+}$ (for example, see O'Connell *et al*., 2004b).

So far only one TnC isoform from anuran muscle, similar in structure and $Ca^{2+}$ -binding properties to the rabbit TnC-f, has been purified and sequenced. However, single fibre studies have shown inter-fibre differences with respect to contractile activation characteristics, which suggests that anuran striated muscle expresses more than one TnC isoform. Thus, the main aims of the present study were (i) to definitively establish whether anuran striated muscle expresses more than one TnC isoform, and if so (ii) to examine the relationship between the myosin heavy chain (MHC) and TnC isoform expression in anuran muscle fibres and (iii) to characterise the anuran TnC isoforms according to the $Sr^{2+}$- and $Ca^{2+}$-activation properties conferred to the single fibres in which they are found.

Adult (body weight 250-380 g) cane toads (*Bufo marinus*) were killed by double pithing in accordance with procedures approved by Victoria University AEEC. The TnC isoform composition of cardiac muscle and of 198 single fibres from the rectus abdominis muscle was investigated using a recently developed method for the unequivocal identification of TnC isoforms on SDS-polyacrylamide gels (O'Connell *et al*., 2004a). The same single fibres were also analysed for their MHC isoform content using the alanine-SDS-polyacrylamide gel electrophoresis protocol of Goodman *et al*. (2003). For a subpopulation of 15 fibres, the $Sr^{2+}$ - and $Ca^{2+}$ -activation characteristics were measured and related to the TnC isoform present.

Our results show that like mammalian striated muscle, the anuran striated muscle expresses two TnC isoforms which can be distinguished electrophoretically. The slowest migrating TnC isoform (TnC-t) was detected in all fibres displaying only twitch MHC isoforms, regardless of their number or identity; the other (TnC-T/c) was detected in fibres displaying the slow-tonic MHC isoform and in cardiac muscle. Fibres containing the TnC-T/c isoform were found to be ~47 times more sensitive to $Sr^{2+}$ and ~3 times more sensitive to $Ca^{2+}$ than fibres containing the TnC-t isoform. From these data we conclude that both anuran and mammalian striated muscle contain two TnC isoforms that play an important role in determining the contractile activation characteristics of the fibres in which they are expressed.

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