Unexpected redundancy between β-adrenoceptor subtypes in early muscle regeneration

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Skeletal muscles can be injured by myriad insults that compromise their functional capacity. Regenerative processes are often slow and incomplete, and so developing novel therapeutic strategies to enhance muscle regeneration represents an important research area. We have shown previously that the β-adrenoceptor (AR) signalling pathway plays an important role in skeletal muscle regeneration after injury (Beitzel *et al.*, 2004; 2007), and that transgenic mice lacking both β_1- and β_2-ARs have delayed regeneration following myotoxic injury (Sheorey *et al.*, 2008, Church *et al.*, 2010). In the present study we further investigated the relative contribution of individual β-AR subtypes to the early stages (up to 7 days) of muscle repair after injury.

Mice (8-9 weeks) lacking β_1-adrenoceptors (β_1-AR KO), β_2-adrenoceptors (β_2-AR KO), or both subtypes of β-adrenoceptors (β_1/β_2-AR KO), were obtained from The Jackson Laboratory (Bar Harbour, ME, USA). Littermate wildtype mice were used as controls for the β_1-AR KO and β_2-AR KO mice, while control mice for the β_1/β_2-AR KO mice were from a C57BL/6 background, as employed previously (Sheorey *et al.*, 2008). Mice were anaesthetized (ketamine 80mg/kg and xylazine 10mg/kg; i.p.) such that they were unresponsive to tail or toe pinch, and the *tibialis anterior* (TA) muscle of the right hindlimb was injected with the myotoxin, Notexin (1μg/ml, i.m.) to cause complete muscle fibre degeneration. Mice were allowed to recover for 7 days, after which TA muscle function was assessed *in situ* as reported previously (Gehrig *et al.*, 2010). Briefly, mice were anaesthetized (60 mg/kg, sodium pentobarbital, i.p.), the right TA muscle was surgically exposed, and the distal tendon was attached to the lever arm of a force transducer, with the knee and foot immobilized. At the conclusion of the experiment mice were killed by cardiac excision while still anaesthetized deeply.

As reported previously (Church *et al.*, 2010), when muscle function was examined in uninjured TA muscles, both β_2-AR KO mice and β_1/β_2-AR KO mice produced significantly less force (Po) than controls ($P < 0.05$), and TA muscles from β_1-AR KO mice showed no significant deficit in force production. At 7 days post-injury, the regenerating TA muscles of β_1/β_2-AR KO mice produced significantly less force than those of controls ($P < 0.05$) but neither β_1-AR KO nor β_2-AR KO mice showed any delayed restoration of force producing capacity. When force production was normalized to cross-sectional area (sPo), or to that of uninjured controls (to account for altered force production between the strains), a similar result was obtained, with the β_1/β_2-AR KO mice displaying delayed restoration of function while mice lacking either β_1- or β_2-adrenoceptors alone did not.

These results suggest that while the absence of both β_1- and β_2-adrenoceptors can delay muscle regeneration after injury, the absence of either subtype alone does not. This apparent redundancy in the β-AR signalling pathway in skeletal muscle is unexpected, and may have important implications for the use of β-AR agonists to enhance regeneration after injury, as well as in the treatment of other conditions where muscle wasting and weakness are indicated.

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