Role of β-adrenoceptors during early skeletal muscle regeneration in mice

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Skeletal muscles can be injured via numerous physical, metabolic and thermal insults, leading to a loss of force production. A greater understanding of the processes controlling skeletal muscle regeneration may lead to novel treatments for muscle injuries and muscle diseases. We have previously identified the β-adrenoceptor (β-AR) signalling pathway as a potential regulator of muscle regeneration after injury (Beitzel *et al*. 2004; 2007). The aim of this study was to determine whether β-ARs are necessary for successful muscle fibre regeneration. Since transgenic mice lacking both β_1- and β_2-AR subtypes are available (*Adrb1*$^{-/-}$*/Adrb2*$^{-/-}$), we determined the physiological role of β-AR signalling during skeletal muscle regeneration after injury. We tested the hypothesis that mice homozygous null for β_1-ARs and β_2-ARs (*Adrb1*$^{-/-}$*/Adrb2*$^{-/-}$) would exhibit impaired muscle regeneration, as evidenced by reduced muscle function.

To examine the role of β-ARs in muscle regeneration, we utilized 7-8 week old *Adrb1*$^{-/-}$*/Adrb2*$^{-/-}$ mice (*n* = 27, Jackson Laboratory, USA, $Adrb1_{tm1Bkk}Adrb2_{tm1Bkk/J}$, stock no. 003810) and C57BL/6 wild-type mice (WT, *n* = 31, Animal Resource Centre, Canningvale, WA, Australia). Mice were anaesthetized deeply (ketamine 76 mg/kg and xylazine 10 mg/kg; *i.p.*) and the extensor digitorum longus (EDL, fast-twitch) muscle was surgically exposed and injected with Notexin (1μg/mL; Lotaxan, Valence, France) to cause complete degeneration of all muscle fibres (Plant *et al*. 2006). The contralateral EDL muscle served as the uninjured control in each case. Mice were allowed to recover for 7, 14 or 21 days, at which time they were anaesthetized deeply with sodium pentobarbitone (60 mg/kg; *i.p.*) and isometric contractile properties of injured and uninjured muscles were determined *in vitro*, as described previously (Plant *et al*. 2004). Mice were killed by cardiac excision while anaesthetized.

Maximum force was not different between uninjured EDL muscles from *Adrb1*$^{-/-}$*/Adrb2*$^{-/-}$ and WT mice ($234 \pm 5 kN/m^2$ and $238 \pm 7 kN/m^2$, respectively). The force producing capacity of muscles from WT mice at 7, 14 and 21 days post-injury was 31%, 64% and 78% of their uninjured control values, respectively. In contrast, the force producing capacity of muscles from *Adrb1*$^{-/-}$*/Adrb2*$^{-/-}$ mice at 7, 14 and 21 days post-injury was 3%, 64% and 78% of their uninjured controls, respectively.

Our findings indicate that while β-ARs are not required for successful skeletal muscle regeneration, as evidenced by the restoration of force producing capacity to uninjured control levels by 14 and 21 days post-injury, they may play an important (and previously unreported) role during early muscle regeneration. Clarification is required on the role of β-AR signalling on inflammatory and early myogenic processes such as myoblast differentiation and cell fusion. Our results suggest that manipulation of the β-AR signalling pathway during early regeneration after injury may improve the rate, extent and efficacy of these regenerative processes. The findings have important implications for the treatment of muscle wasting conditions especially muscle diseases where regeneration is defective.

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