Intramuscular administration of formoterol attenuates loss of muscle mass and function after denervation of the rat masseter muscle

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β-adrenoceptor agonists (β2-agonists) are used commonly for treating bronchospasm, but some have powerful muscle anabolic properties with potential therapeutic application for muscle wasting conditions. Manipulating β-adrenoceptor signalling can affect the pathways responsible for hypertrophy and atrophy of skeletal muscle (Lynch & Ryall, 2008) and intramuscular (i.m.) administration of the β2-agonist, formoterol, can increase mass and force-producing capacity of regenerating rat skeletal muscles after myotoxic injury (Ryall *et al.*, 2008). Masticatory muscles, particularly the masseter, are subject to severe atrophy as a consequence of orthodontic surgery, developmental and neuromuscular disorders. These changes in muscle size and function can influence the timing and nature of orthodontic procedures and rehabilitation. Therefore attenuating muscle wasting and weakness of the orofacial muscles has important clinical relevance. We tested the hypothesis that i.m. administration of formoterol could attenuate atrophy and weakness of rat masseter muscles after denervation.

Male Sprague Dawley rats (4 weeks of age) were assigned into four groups: surgical sham + saline; denervated only; denervated + formoterol; or formoterol-only. Rats were anaesthetized with ketamine (225 mg/kg; i.p.) and xylazine (30 mg/kg; i.p.) with supplemental doses to maintain a depth of anaesthesia, such that there was no response to either tail or toe pinch. The denervated groups had their left masseteric nerve surgically excised. Formoterol (100 μg in 0.1 ml saline) was injected into the left masseter muscle every 3 days in the formoterol treated groups for 8 weeks. For all i.m. injections, rats were anaesthetized by ventilation of 5% isoflurane (1 ml/ml) and the depth of anaesthesia maintained by isoflurane delivery (0.5 l/min) to a nose cone placed over the rat's snout.

At the end of the 8 week denervation/treatment protocol, the rats were anaesthetized deeply with ketamine and xylazine (as described) and muscle architecture and volume determined using magnetic resonance imaging (Bruker Biospec 4.7 Tesla, MRI/MRS, Germany). The chest was then opened and the rats killed by rapid cardiac excision. Masseter muscles were carefully excised, blotted, weighed and stored for histological, functional (single permeabilized fibres), and biochemical assessments using techniques described in detail previously (Schertzer *et al.*, 2008). Denervation resulted in a 38% decrease in masseter muscle mass and volume ($P<0.05$) which was attenuated by i.m. administration of formoterol, which resulted in the mass of the masseter muscle decreasing by 24% in the denervated + formoterol group. Administration of formoterol alone increased masseter muscle mass by 36% ($P<0.05$). In single permeabilized fibres, denervation resulted in a decrease in absolute but not specific force and a decrease in the sensitivity of the contractile apparatus to Ca^{2+}. Although i.m. formoterol treatment prevented all denervation-induced changes in masseter muscle mass and fibre cross-sectional area, it did not prevent the decrease in Ca^{2+} sensitivity in single fibres. Denervation caused an increased expression of slow isoforms of troponin I, T and C, which was prevented by i.m. formoterol treatment.

The finding that i.m. administration of formoterol can attenuate denervation-induced changes in masseteric muscles identifies its potential clinical application for orthodontics in maintaining muscle structure and function during surgical (and other) interventions for orofacial disorders.

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