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Acute effect of androgens on maximal force-generating capacity and electrically evoked calcium transient in mouse skeletal muscles

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ABSTRACT

As androgens might have rapid androgen-receptor (AR) independent action on muscle cells, we analysed the *in vivo* acute effect of androgens on maximal force generation capacity and electrically evoked calcium transient responsible for the excitation–contraction coupling in skeletal muscle from wild-type male mice and muscle fibre androgen receptor (AR) deficient ($AR^{skm-/y}$) male mice. We tested the hypothesis that acute *in vivo* androgen treatment improves contractility and modifies calcium transient in mouse hindlimb muscles. In addition, we determined whether the reduced maximal force generation capacity of $AR^{skm-/y}$ mice is caused by an alteration in calcium transient. We found that acute dehydrotestosterone (DHT) and testosterone treatment of mice does not change *in situ* maximal force, power or fatigue resistance of tibialis anterior muscles. In agreement with this observation, maximal force and twitch kinetics also remained unchanged when both whole extensor digitorum longus (EDL) muscle or fibre bundles were incubated *in vitro* with DHT. Electrically evoked calcium transient, i.e. calcium amplitude, time to peak and decay, was also not modified by DHT treatment of EDL muscle fibre bundles. Finally, we found no difference in calcium transient between $AR^{skm-/y}$ and wild-type mice despite the reduced maximal force in EDL fibre bundles of $AR^{skm-/y}$ mice. In conclusion, acute androgen treatment has no ergogenic effect on muscle contractility and does not affect calcium transient in response to stimulation. In addition, the reduced maximal force of $AR^{skm-/y}$ mice is not related to calcium transient dysfunction.

1. Introduction

Testosterone, a male sex steroid hormone that is converted to dihydrotestosterone (DHT), notably in skeletal muscle, binds the androgen receptor (AR) in the cytosol. This complex translocates into the nucleus where it either activates or represses target genes. The expression of many genes is modified within hours to days after androgen exposure. Chronically administrated androgens can exert an ergogenic action, i.e. increases skeletal muscle maximal force production via muscle growth. It has also been reported that androgens can exert fast actions that are thought to be too rapid to be explained by this process [1,2]. For example incubation of fast skeletal muscle fibre bundles isolated from mice of both sex with DHT at 630 μg/ml for 30 min increases maximal force production by 24–30% [3]. Moreover, treatment of such fibre bundles with an AR inhibitor did not block DHT-induced maximal force increase, suggesting that this effect of DHT does not involve AR signaling. Several other studies also support the possibility of signal transduction pathways independent of AR signalling [2–9]. In the present study we would like to confirm if this rapid response to acute androgen treatment is observed in skeletal muscle *in vivo*. If an increase in maximum force is observed we will then look at the effect on calcium handling since this rapid AR response does seem to not involve AR signalling. Calcium is known to play a crucial role in skeletal muscle physiology, and several lines of evidence indicate that androgens modulate calcium homeostasis [2,10–12]. Chronic androgen administration in orchidectomized rodent has been shown to interfer with many aspects of calcium release and uptake of the sarcoplamic reticulum of the myocardium [11,12], responsible for the excitation–contraction–relaxation cycle.

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Moreover, it was recently shown that acute testosterone treatment increases intracellular calcium in myotubes [4–6]. However, acute effects of androgens on calcium transient initiating the contraction of the skeletal muscle are poorly characterised. It is possible that the reduced maximal force generation capacity that was observed in skeletal muscle from $AR^{skm-/y}$ mice, in which the AR was selectively ablated in skeletal muscle fibres [13], is related to an alteration of the calcium transient. The selective ablation of AR in muscle fibres from $AR^{skm-/y}$ mice leads to a 20–25% reduction in the maximal force generation capacity in mouse fast muscle, with no change in muscle weight [13].

The main purpose of this study was to examine the acute effect of androgens on the skeletal muscle of wild-type (WT) and $AR^{skm-/y}$ mice. To our knowledge, the acute effect of androgens *in vivo* on muscle performance has not yet been studied. Our first hypothesis was that maximal force would be increased by *acute in vivo* androgen administration in both WT and $AR^{skm-/y}$ mice. A second hypothesis is that electrically evoked calcium transient would be affected by acute androgen administration in mice. A third hypothesis is that reduced force generation capacity of the $AR^{skm-/y}$ mice would be related to altered calcium transient. Such findings would be of benefit to antidoping agencies, as they would indicate that acute androgen administration might have ergogenic effects in athletes. Moreover, they would open new avenues to develop treatments to combat the muscle weakness observed in different contexts (ageing, neuromuscular diseases, etc.).

2. Materials and methods

2.1. Animals

All procedures were performed in accordance with national and European legislations and all experimental protocols have been approved by the French Departmental Direction of Animal Protection (agreement 75-1102). Male CD1 mice (referred to below as WT), $AR^{skm-/y}$ mice [13] and age-matched control (referred to below as WT) mice were analysed. Testosterone and DHT (Sigma, L'Isle d'Abeau Chesnes, France) treatments are described below.

2.2. In situ TA muscle contractility

We analysed *in situ* tibialis anterior (TA) muscle contraction in response to nerve stimulation as described previously [14,15]. Mice were anesthetized using pentobarbital (60 mg/kg ip). Body temperature was maintained at 37 °C using radiant heat. The knee and foot were fixed with pins and clamps and the distal tendon of the muscle was attached to a lever arm of a servomotor system (305B, Dual-Mode Lever, Aurora Scientific) using a silk ligature. The sciatic nerve was proximally crushed and distally stimulated by a bipolar silver electrode using supramaximal square wave pulses of 0.1 ms duration. Absolute maximal force (P0) generated during isometric contractions in response to electrical stimulation (frequency of 75–150 Hz, train of stimulation of 500 ms) was determined at L0 (length at which maximal tension was obtained during the tetanus). Absolute maximal power (Pmax, mW) was calculated from force–velocity data. The isometric contraction was initiated during the first 300 ms, then muscle shortening was allowed during the last 200 ms. Five shortening contractions (15–50% of P0) were performed, each separated by a 45 s rest period. The corresponding shortening speeds were measured during the first 50 ms. Finally, the fatigue resistance was assessed. The fatigue resistance protocol consisted of 40 contractions (100 Hz for 300 ms, evoked once every second), and the force produced at the end of the protocol was measured (% of initial force).

2.3. In vitro EDL muscle contractility

Isometric contractile properties of extensor digitorum longus (EDL) muscles were studied *in vitro*. Measurements were performed according to methods previously detailed [16]. Briefly, muscles were dissected and connected at one end to an electromagnetic puller and at the other end to a force transducer. Muscles were dissected free from adjacent connective tissue and soaked in an oxygenated Tyrode solution (95% O_2 and 5% CO_2) containing (mM): 118 NaCl, 25 $NaHCO_3$, 5 KCl, 1 KH_2PO_4, 2.5 $CaCl_2$, 1 $MgSO_4$, 5 glucose, maintained at a temperature of 20 °C. After equilibration (45 min), stimulation (frequency of 125 Hz, train of stimulation of 300 ms) was delivered through electrodes running parallel to the muscle. P0, the absolute twitch force (Pt), the contraction time and the half-relaxation time during the twitch were measured.

2.4. In vitro EDL fibre bundle contractility and calcium transient

EDL muscles were pinned in SYLGARD®coated dishes containing normal physiological solution (in mM: 148 NaCl, 4.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonicacid], and 5.5 glucose (pH = 7.4). Small bundles of 10–15 fibres arranged in a single layer were dissected lengthwise, tendon-to-tendon, with the use of microscissors. Muscle preparations were loaded during 1 h with 3 μM of the Fura-2 AM fluorescent calcium probe. After Fura-2 loading, muscle fascicles were mounted in an experimental chamber allowing isometric force and cytosolic calcium measurement and electrical stimulation. One extremity was attached to a hook linked to a micromanipulator and the other extremity was fixed to an electromagnetic force-transducer device described elsewhere [17]. Muscle preparations were stretched until the twitch amplitude elicited by efficient electrical stimuli (5 ms duration; 14 V; 0.2 Hz) reached the maximum value. The diameter of each contracting fibre was measured to calculate the cross sectional area. The sum of the areas was used to calculate the cross-sectional area (mm^2) and to normalise force amplitude (mN). Electrical triggered force development was acquired under two stimulus protocols: (1) 5 ms duration/14 V/0.2 Hz, protocol designed to elicit single separated twitches (contraction related to a unique action potential); (2) 20 pulses of 5 ms duration/14 V/100 Hz, protocol designed to elicit perfect tetanus. The evaluation of the contractile responses was done by measuring the amplitude (mN/mm^2), the time to peak (ms) and the time constant of relaxation (s^{-1}) of the twitch, and the amplitude (mN/mm^2) and the time constant of relaxation (s^{-1}) of the tetanus. All experiments were conducted at room temperature.

Ratiometric Fura-2 fluorescence measurements were made using optical excitation filters of 380 and 360 nm and an IonOptix microStepper Switch integrated system. Emitted fluorescence (510 nm) was background subtracted. Cytosolic [Ca] was calculated at rest according to a modified method from Grynkiewicz and collaborators [18]. A pseudo ratiometric approach was used to acquire calcium transients. Preparations were electrically stimulated with a pace protocol (0.5 Hz; 4 ms duration; 14 V) designed to induce a single action potential. Fura-2 fluorescence was first recorded at 1000 Hz under 380 nm excitation during 10 electrical stimulations. Thereafter, Fura 2 fluorescence was recorded at 360 nm. The 10 records were averaged at each excitation wavelength. Finally the calcium transient was calculated by making the ratio of the means (360/380) and transformed in [Ca] values using the method described for resting [Ca] determination. The time to peak, the amplitude and the rate constant of the calcium decay (by fitting a monoexponential on the recovery phase of the calcium transient) were calculated.