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Effect of denervation on the androgen-induced expression of actin and CPK mRNAs in the levator ani muscle of the rat

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In the adult male rat, the castration-induced atrophy of the levator ani (LA) muscle was found to be associated with a decrease in the relative levels of both actin and creatine phosphokinase (CPK) mRNAs. The typical recovery of these two sequences following 5 days of testosterone propionate (TP) replacement therapy was not impaired by the bilateral denervation of the LA. This indicated that TP was the sole trophic factor regulating the plasticity of these two mRNAs and challenged the hypothesis that androgen action might be neuronally mediated. The observation that denervation led to a severe repression of both actin and CPK messages only in the absence of TP replacement therapy suggested that the nerve impulse could play an accessory role in the control of their expression.

Androgen action; Protein synthesis; Perineal muscle; Denervation

1. INTRODUCTION

The so-called levator ani (LA) muscle of the male rat is exquisitely sensitive to androgens. As opposed to skeletal muscle, protein synthesis activity in the LA is greatly reduced after castration and restored after testosterone administration [1]. The castration-induced atrophy of the LA results in important ultrastructural changes [2] and alterations in its contractile properties [3]. Using specific cDNA probes, we recently demonstrated that the observed decrease in protein synthesis after 10 days of androgen withdrawal might be transcriptionally regulated and involved a decrease in the relative abundance of mRNAs encoding muscle actin and creatine phosphokinase (CPK). Since a decrease in muscle actin was observed in different models of denervation atrophy [9,16] and that CPK mRNA is up-regulated in the presence of spinal cord complex in vitro [17], we therefore postulated that the androgen withdrawal might have impaired the motoneuron activity of the LA, which could be related to some extent to the observed repression of both mRNAs.

We report that in castrated rats, the expression of those two androgen-sensitive mRNAs of the LA can also be induced in denervated muscle in response to testosterone replacement therapy. This indicates a direct action of the hormone on the muscle plasticity and suggests an accessory role of the muscle innervation on the expression of these selected mRNAs.

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2. EXPERIMENTAL

Castration (GDX)-induced atrophy of the LA was induced in adult male Sprague-Dawley rats (250 g) for a period of 7 days. The GDX animals were then separated in several groups. One of the groups was left without further treatment (GDX) and immediately sacrificed, while one received intraperitoneal injections of testosterone propionate (1 mg/day) for 5 days (GDX + TP). In a third group, bilateral denervation of the LA/BC complex was performed prior to TP replacement therapy (GDX-DEN + TP). A fourth group of GDX rats was submitted to denervation without subsequent injections of TP (GDX-DEN). In order to control for non-specific vascular influences due to the surgical procedure, a sham denervation was performed in animals receiving TP treatment (GDX-shamDEN + TP). At the end of the TP treatment, the animals were killed by decapitation and the LA muscle was excised, weighed and immersed in 4 M guanidine isothiocyanate for the subsequent isolation of RNA as described [8].

Qualitative assessment of the level of both actin and CPK mRNA expression for each group was carried out by Northern analysis using 5 μg of total RNA obtained from the LA muscle as described [9]. The labelling of the 1.1 kb actin cDNA probe was performed using a multiprime labelling system (Amersham) which is based on a technique originally described by Feinberg and Vogelstein [10]. The CPK mRNA was probed using a synthetic oligonucleotide complementary to the 3′ non-coding region of muscle CPK mRNA [11] and was end-labelled with T_4 polynucleotide kinase as previously described [12]. Detection of the hybridization signal by autoradiography was carried out as described [9] and quantitation of the signal on the film was achieved by digital imaging.

3. RESULTS

Seven days following orchiectomy, the LA wet weight of the rats was significantly reduced reaching about 50% of the control value and treatment with replacement doses of TP (GDX + TP) for 5 days restored the initial weight of the muscle (fig.1). When

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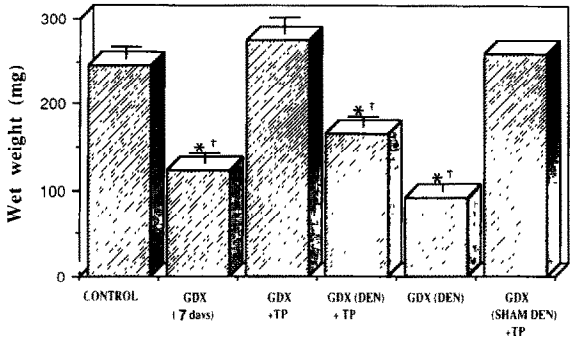

Fig.1. Levator ani (LA) wet weight at the time of sacrifice in adult male rats. Castration-induced atrophy of the LA was allowed to take place for 7 days and a group of rats was then sacrificed for LA weight determination and preparation of total cellular RNA (GDX-7 days). The remaining GDX animals (n = 16) were separated into four groups to establish the effect of the pudendal nerve section on the LA recovery as described under section 2. Values are mean ± SE, n = 4 determinations/group. *, statistical significance from control group ($P < 0.05$), †, statistical significance from the GDX + TP group ($P < 0.05$).

denervation was performed prior to the TP treatment of the same duration (GDX-DEN + TP), the recovery of muscle mass was found to be incomplete and significantly different from the GDX + TP group. Sham denervation did not alter the muscle response to TP treatment indicating that the partial recovery observed in GDX-DEN + TP was specifically induced by the nerve section. When TP replacement therapy was omitted, denervation induced a further decrease in muscle of GDX rats (GDX + DEN, fig.1).

To verify whether the modifications in muscle mass could involve similar changes in the LA gene expression, muscle actin and CPK mRNA were probed for each of the experimental groups. Hybridization signals and the corresponding quantitative evaluation are presented in panels A and B, respectively (figs 2 and 3). As shown in fig.2, the hybridization signal specific for the actin message displays the typical 75% reduction observed 7 days after castration in the LA muscle (fig.2b) compared to control value (fig.2a). Although the actin mRNA was partially recovered in the GDX + TP group (fig.2c), it is clear that denervation of the muscle prior to TP treatment did not impair the recovery of actin mRNA (fig.2d). In fact, the recovery in the specific message reached 70% of the control values in c and d. Interestingly, the absence of TP replacement therapy in the GDX-DEN group severely reduced actin mRNA levels since the hybridization signal was found to be virtually absent (fig.2e). Such an important decrease is not obtained when castration alone is allowed to proceed for several weeks (not shown). The TP response in the sham-denervated group (GDX-shamDEN + TP, fig.3f) was equivalent to the GDX + TP group. The assessment of the CPK mRNA expression for the same experimental groups indicated a pattern of response similar to that found for actin mRNA. Although castration did not decrease the expression of the CPK message to a similar extent (fig.3b) compared to control (fig.3a), the partial recovery following TP administration (fig.3c) was still not impaired by denervation (fig.3d). Again, the absence of TP treatments in GDX + DEN led to an im-

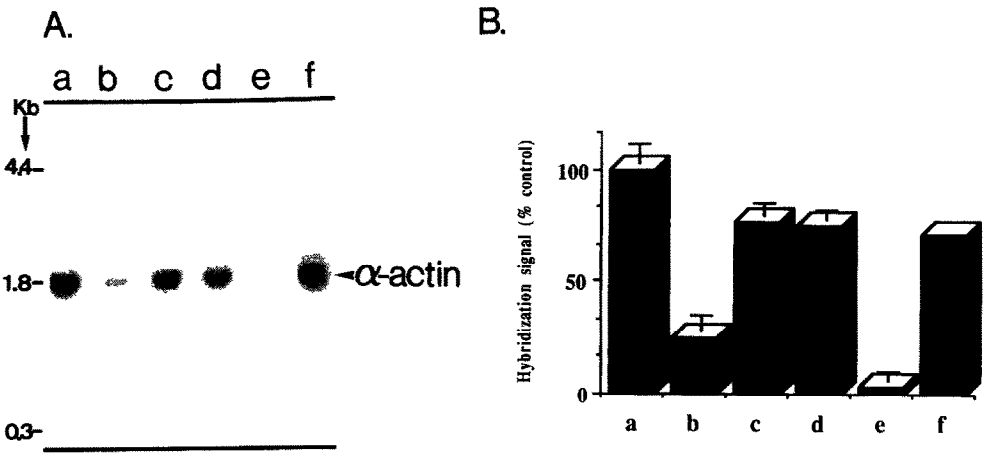

Fig.2. Northern blot analysis of RNA samples for the detection of actin mRNA. 5 μg of total cellular RNA from each group were heat-denatured in 50% formamide and 6% formaldehyde by incubating for 5 min at 65°C. The RNA was then electrophoresed on a 1.5% agarose gel containing 2% formaldehyde at 90 V for 3 h. The gel was stained with ethidium bromide and destained in tap water before the RNAs were transferred by capillarity to Hybond-N hybridization transfer membranes. The labelling of the actin cDNA probe was performed using a multiprime labelling system (Amersham) and [^{32}P]dCTP (3000 Ci/mmol) as labelled nucleotide [10]. Prehybridization, hybridization and washing of the membranes were performed as described [9]. The membranes were exposed at −80°C to Kodak X-Omat film with intensifying screens for the visualization of the hybrids. Panel A shows a representative experiment: a, control; b, GDX-7 days; c, GDX + TP; d, GDX-DEN + TP; e, GDX-DEN; f, GDX sham DEN + TP. Panel B: The relative intensity of the bands on the films was routinely quantified using a digital imaging system (Research Analysis System, Amersham). Values are mean ± SD for n = 3 in all cases. Because of the small sample size, no attempt for statistical analysis was made.

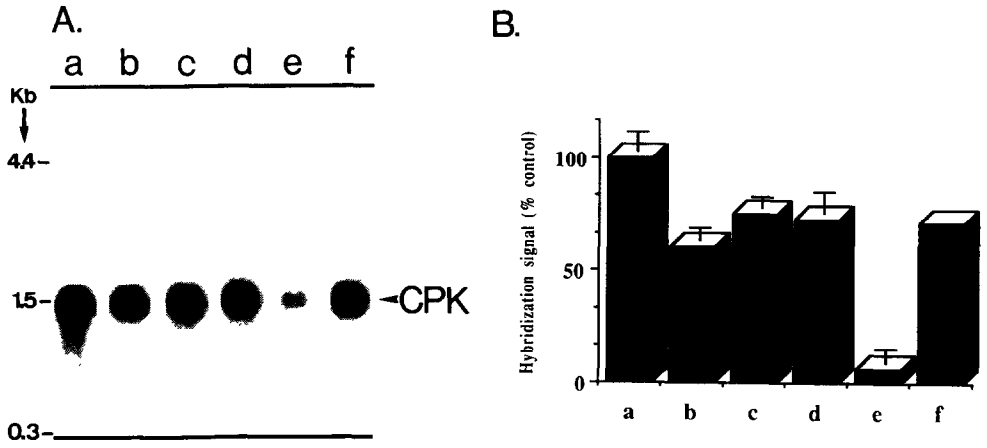

Fig.3. Northern analysis of RNA samples for the detection of CPK mRNA (Panel A). 5 μg of RNA for each group was size fractionated by electrophoresis and transferred to the hybridization membrane as described in fig.2. A 21-mer oligonucleotide probe complementary to the 3′ non-coding region of the CPK mRNA was synthesized using a Bio Search DNA synthesizer (Model 8700) and further purified by electrophoresis. The end-labelling of the probe was carried out using T_4 polynucleotide kinase to a specific activity of $1-3 \times 10^8$ cpm/μg [12]. Prehybridization, hybridization and washing of the membrane were performed essentially as described [18]. Codification of the experimental group is as described for fig.2. (Panel B) Digital imaging quantitation of the hybridization signal. Values are mean ± SD for $n = 3$ in all cases. Because of the small sample size, no attempt for statistical analysis was made.

portant decrease in the expression of the message (fig.3e). The extent of recovery in the sham-denervated group (fig.3f) was found to be equivalent to the GDX + TP group.

4. DISCUSSION

Based on the expression of two selected sequences, we attempted to demonstrate whether the nerve impulse was required to mediate the effects of testosterone on the LA muscle. In castrated rats, we observed a partial recovery of the LA mass in response to TP treatment when the muscle was previously denervated. This indicated that the nerve impulse was necessary for the muscle to fully respond to the TP replacement therapy. As reported previously, denervation of the LA induced a shift toward a more oxidative metabolism and a decrease in myofibrillar proteins [13]. Those modifications are not found in castration-induced atrophy which suggests that androgens and the nerve impulse exert their trophic influences via different metabolic pathways and should both be required for the muscle to fully restore its functional integrity.

In a previous study, we demonstrated that the relative amount of both actin and CPK mRNAs were reduced in castration-induced atrophy of the LA and raised the question whether androgens were the sole trophic factors regulating their plasticities. According to the present report, the induction of actin and CPK messages in response to TP treatment took place in the absence of nerve impulse; this indicates that the denervated muscle still behaves like an androgen target tissue and that, in castrated rats, these selective determinants of the LA protein synthesis depend primarily on testosterone for their expression. Such a result is at variance with the experiment of Buresova et al. [7] which dealt with total protein synthesis. In that case, denervation of the LA was found to completely abolish the increase in protein synthesis in response to testosterone replacement therapy.

In the absence of testosterone, however, the effect of denervation was shown to be important since the relative abundance of actin and CPK mRNAs was then severely decreased to levels never attained by a castration-induced atrophy of comparable duration. Therefore one can postulate a hierarchy between the endocrine and neural stimulus for the expression of CPK and actin mRNAs in the LA. We have shown that androgens represent the major determinant of the expression of these two sequences whereas the nerve impulse contributes to maintain the remaining levels of those specific messages once androgens are withdrawn.

In contrast to the present finding, several data suggested that testosterone effects in the LA were neuronally mediated. Castration was shown to induce a loss of total acetylcholine receptors (AChR) in this muscle [4] as well as a marked decrease in the total acetylcholinesterase activity [5]. Even though the effect of testosterone on AChR is not yet clearly established, a specific accumulation of the labelled testosterone in motoneurons of the spinal nucleus of the LA (SN) was already demonstrated [6]. These findings strongly support the hypothesis that the SN motoneurons were under the control of androgens and that all the trophic influences of androgens in the LA might be neuronally mediated, as suggested by Bleish et al. [4]. However, our results clearly demonstrate a direct action of testosterone therefore challenging the possibility that

the trophic action of androgens in the LA acts exclusively by the potentialisation of motoneurons activity. Such a direct action implies the need for androgen receptors which have been demonstrated in the sarcoplasma of LA muscle [14]. It is therefore postulated that the androgen-controlled plasticity of the LA involves the well-established molecular mechanism of gene regulation by steroid hormones [15].

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