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CHANGES IN CONTRACTILE PROPERTIES BY ANDROGEN HORMONES IN SEXUALLY DIMORPHIC MUSCLES OF MALE FROGS (*XENOPUS LAEVIS*)

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SUMMARY

1. Male frogs (*Xenopus laevis*) were castrated then given either empty or testosterone-filled implants to produce animals with low or high levels of circulating testosterone. Eight weeks later the contractile properties of an androgen-sensitive forelimb flexor, the flexor carpi radialis muscle (FCR), were measured *in vitro*. Another forelimb flexor muscle, the coracoradialis, and a hindlimb muscle, the iliofibularis, were analysed similarly.

2. Plasma testosterone levels were $0{\cdot}9 \pm 0{\cdot}3$ ng/ml ($\pm$S.E.M.) in castrated frogs with blank implants (C) and $61{\cdot}3 \pm 4{\cdot}7$ ng/ml in castrates with testosterone implants (CT). Unoperated males, sampled at various times of the year, ranged between 10·8 and 51·0 ng/ml.

3. With direct electrical stimulation of the FCR, contraction time of the isometric twitch was not affected by testosterone levels. Relaxation times were affected, however. Half- and 90% relaxation times were 27 and 42% longer, respectively, for CT compared to C muscles.

4. Testosterone also had no effect on the contraction time of twitches elicited by stimulation of the FCR nerve. Half- and 90% relaxation times were 51 and 76% longer, respectively, for CT compared to C muscles.

5. Tetanus tension, elicited by direct stimulation of the FCR at 50 Hz, was 86% greater in CT compared to C muscles. The average cross-sectional area of FCR muscle fibres was 84% greater in CT muscles. These results implied that testosterone treatment had no effect on specific muscle tension.

6. Stimulation of the FCR nerve at 50 Hz resulted in 53% less tension than the same stimulus applied directly to CT muscles. In C muscles the difference was only 14%. This suggested that testosterone treatment reduced synaptic efficacy.

7. In CT muscles, direct or nerve stimulation of fibres in the shoulder region of the FCR elicited twitches that contracted and relaxed more slowly than fibres in the

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elbow region. In C muscles there was no difference in contraction or relaxation time between fibres in the shoulder and elbow regions.

8. Testosterone treatment had little effect on contraction and relaxation times or tension levels of coracoradialis or iliofibularis muscles.

INTRODUCTION

During the mating behaviour known as amplexus, male frogs use their forelimb flexor muscles to clasp females for hours or even days without interruption. For much of this time, males clasp females loosely, generating a constant low level of tension to maintain position. If the female moves suddenly, as she does in an escape response or when going to the surface for air, the male tightens his grip to avoid being dislodged (Hutchison & Poynton, 1963). The forelimb flexors of frogs are sexually dimorphic. Muscles are larger and contract more slowly in males than in females (reviewed in Herrera & Regnier, 1991). A primary effector of clasping behaviour, the flexor carpi radialis muscle, is sensitive to androgens in adult male frogs. Experimental manipulation or seasonal changes in testosterone levels affect the size and contractile kinetics of flexor carpi radialis muscles. Muscles grow larger and contract more slowly in response to high testosterone levels (Muller, Galavazi & Szirmai, 1969; Melichna, Gutmann & Stichova, 1972; Thibert, 1986; Regnier & Herrera, 1989, 1990). There is also some evidence that testosterone enhances electrical excitability in the clasping reflex pathway (Erulkar, Kelley, Jurman, Zelman, Schneider & Kreiger, 1981).

To understand better the cellular mechanisms of androgen action on sexually dimorphic motor systems, we have more fully characterized the effects of testosterone on forelimb flexor muscles and neuromuscular junctions. The contractile properties of the flexor carpi radialis and another forelimb flexor, the coracoradialis muscle, were measured in male *Xenopus laevis* with high and low levels of testosterone. Contractile properties of the iliofibularis, a leg muscle, were also measured for comparison. Preliminary reports of this work have been published (Regnier & Herrera, 1989, 1990; Herrera & Regnier, 1991).

METHODS

Male *Xenopus laevis* (7·0–7·9 cm body length, 38–50 g body weight) were obtained from Nasco (Oshkosh, WI, USA) at different times of the year. Frogs were fed with Nasco frog brittle three times a week, and were exposed to a cycle of 12 h light, 12 h dark. All experimental frogs were anaesthetized by immersion in 0·10–0·15% aqueous 3-aminobenzoic acid ethyl ester (Tricaine, Sigma, USA), chilled on ice, then castrated by removing the testes and adherent fat bodies through small abdominal incisions. In half of the castrated frogs, silastic tubes (o.d. 0·65 mm, i.d. 0·3 mm, Dow Corning) packed with 1·0 cm of crystalline testosterone (Steraloids, Wilton, NH, USA) were implanted subcutaneously in the dorsal lymph sac. These animals were referred to as CT, signifying that they were castrated and received testosterone implants. The other half of the castrated frogs were implanted with identical but empty tubes and were referred to as C animals. Muscles from CT and C frogs will be referred to as CT muscles and C muscles, respectively. Following operations, frogs were kept in individual tanks for 8 weeks. Unoperated frogs were used for some experiments.

At the time of acute experiments frogs were anaesthetized by immersion in 0·15% Tricaine, killed by double pithing, and blood was collected from the sciatic vein. Plasma testosterone levels were measured by a highly specific radioimmunoassay (Coat-A-Count Total Testosterone, Diag-

nostic Products, Los Angeles, CA, USA). Nerve–muscle preparations of the flexor carpi radialis (FCR), coracoradialis (CR), and iliofibularis (IL) muscles were dissected and superfused with Ringer solution of composition (mM): NaCl, 116; KCl, 2; $CaCl_2$, 1·8; TES (*N*-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid) buffer, 5; pH 7·2, at 22–24 °C. For each muscle, the tendon of fibre origin was secured by pinning down, in a Sylgard-lined petri dish; the humerus for the FCR, the sternum for the CR and the origin tendon for the IL. The tendon of insertion was attached to an isometric force transducer (Grass FTO3C or Statham UC2). Muscles were adjusted to the lengths that elicited the maximum twitch tension. Measurements were also taken with varying amounts of muscle stretch to look at the relationships between length, tension, and contraction time. Sarcomere spacing was measured from video microscope images of sarcomeres in surface fibres using a Zeiss 40/0·75 water immersion objective at 400 ×. Tension signals were digitized at 1 kHz and analysed off-line (RC Electronics). Contractions were elicited using Pt–Ir wires placed in contact with the muscle for direct stimulation (1–2 ms duration) or by stimulating spinal nerves 2 and 3 separately or together (0·1 ms duration) with suction electrodes. Stimulus voltage was 1·5–3·0 times that needed to elicit a maximal response. For comparison of nerve *vs.* directly elicited muscle contractions, nerve stimulation was followed, after a 2 min rest period, by direct muscle stimulation. To determine if direct muscle stimulation also caused excitation of intramuscular nerve branches of motor nerves, the maximal twitch tension that could be elicited by direct stimulation was compared for four control muscles in the absence *vs.* presence of *d*-tubocurarine (10 μM). The presence of *d*-tubocurarine had no obvious effect on the twitch amplitude of these control muscles indicating that direct muscle stimulation did not cause secondary stimulation of motor nerves. Heglund & Cavagna (1987) have also found no effect of *d*-tubocurarine on the force produced by direct stimulation of rat extensor digitorum longus muscles. Contraction time was measured as the time from the initial development of tension to the peak of the twitch. Relaxation time was measured as the time to relax half or 90% from the peak tension.

Muscles used to measure fibre size were pinned at the length used for tension measurement and frozen whole by immersion in 2-methylbutane cooled with liquid nitrogen. Cross sections (10 μm) were stained for succinate dehydrogenase activity using a modification of the method of Blanco, Sieck & Edgerton (1988). Cross-sectional area was measured for every fibre in each section using video microscopy and image analysis (Analytical Imaging Concepts, Roswell, GA, USA). Total cross-sectional area of shoulder fibre strips was used for estimates of specific tension.

Throughout this paper values are reported as means ± standard errors of the mean. For normally distributed samples, comparisons were done with Student's two-tailed *t* test. Proportions are reported as proportion ± standard error of the proportion and were compared using Mann–Whitney U non-parametric statistics.

RESULTS

Castrated frogs with testosterone implants (CT frogs) exhibited darkly pigmented stripes on the medial surface of the forelimb extending from the elbow to the tips of all digits, similar to breeding males with high testosterone levels. This characteristic was apparent within a week of implantation and persisted until the animals were killed. Castrated frogs with empty implants (C frogs) lacked the pigmented stripes.

Plasma testosterone levels

Plasma testosterone levels were measured for all experimental frogs. The C group had very low testosterone levels (0·9 ± 0·3 ng/ml, $n = 7$), showing that castration effectively eliminated the major source of the hormone. Testosterone levels for the CT group were 68 times higher (61·3 ± 4·7 ng/ml, $n = 7$). For comparison, plasma testosterone levels were also measured in unoperated frogs at various times of the year. Unoperated frogs had testosterone levels intermediate to CT and C frogs and levels varied over a wide range (10·8–50·0 ng/ml, $n = 15$).

TABLE 1. Contractile kinetics of FCR muscles

	Twitch 1 Hz			Stimulus train 10 Hz		Tetanus 50 Hz		
	Contraction time (ms)	Half-relaxation time (ms)	90% relaxation time (ms)	Half-relaxation time (ms)	90% relaxation time (ms)	Contraction time (ms)	Half-relaxation time (ms)	90% relaxation time (ms)
Direct stimulation								
CT	28·0 ± 1·1	25·0 ± 2·3	63·0 ± 6·0	65·0 ± 5·3	230·0 ± 35·6	49·0 ± 1·9	101·0 ± 5·2	330·0 ± 22·4
C	26·0 ± 1·2	20·0 ± 1·6	44·0 ± 2·5*	42·0 ± 6·4*	119·0 ± 16·4	37·0 ± 6·6	80·0 ± 2·5*	288·0 ± 60·7
Nerve stimulation								
CT	22·0 ± 0·8‡	22·0 ± 2·0	60·0 ± 8·5	51·0 ± 4·8	143·0 ± 12·6	91·0 ± 5·7	86·0 ± 8·8	242·0 ± 33·0
C	22·0 ± 0·8‡	14·0 ± 0·8**‡	34·0 ± 2·2**‡	37·0 ± 4·0*	95·0 ± 10·0**	54·0 ± 8·3**	74·0 ± 4·6	226·0 ± 34·1

Values are means ± S.E.M.

*$P < 0·05$ and **$P < 0·01$, differences between CT and C muscles; †$P < 0·05$ and ‡$P < 0·01$, differences between direct and nerve stimulation within CT and C groups.

TABLE 2. Contractile kinetics of CR and IL muscles

	Twitch 1 Hz			Stimulus train 10 Hz		Tetanus 50 Hz		
	Contraction time (ms)	Half-relaxation time (ms)	90% relaxation time (ms)	Half-relaxation time (ms)	90% relaxation time (ms)	Contraction time (ms)	Half-relaxation time (ms)	90% relaxation time (ms)
CR								
CT	24·0 ± 0·7	13·0 ± 0·5	38·0 ± 2·7	39·0 ± 4·6	105·0 ± 13·4	47·0 ± 2·1	102·0 ± 12·3	220·0 ± 26·2
C	22·0 ± 0·1	12·0 ± 0·3	30·0 ± 2·1	28·0 ± 4·5	75·0 ± 11·3	35·0 ± 2·3*	83·0 ± 3·8	182·0 ± 14·2
IL								
CT	26·0 ± 1·4	24·0 ± 1·7	61·0 ± 2·8	52·0 ± 6·8	120·0 ± 21·9	38·0 ± 3·6	68·0 ± 2·4	149·0 ± 19·8
C	28·0 ± 1·0	21·0 ± 2·0	46·0 ± 3·4*	46·0 ± 8·2	91·0 ± 12·3	28·0 ± 2·1*	75·0 ± 4·9	151·0 ± 15·1

Values are means ± S.E.M. Markers for P values are the same as in Table 1.

Effects of testosterone treatment on contractile kinetics

The effects of testosterone on contractile kinetics of directly stimulated FCR muscles are summarized in the top section of Table 1. Twitch contraction times did not differ between CT ($n = 6$) and C ($n = 6$) muscles. Twitch relaxation times were

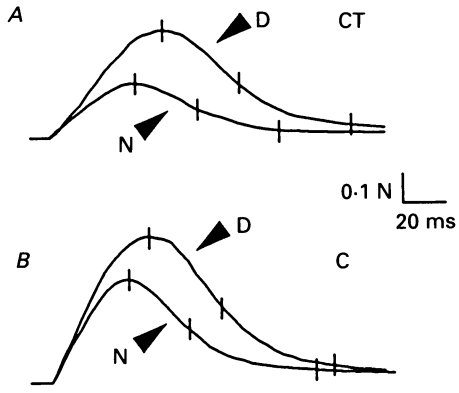

Fig. 1. Twitch tension records from CT (*A*) and C (*B*) FCR muscles stimulated directly (labelled D) and via the nerve (labelled N). Marks in traces indicate peak amplitude, half-relaxation amplitude and 90% relaxation amplitude (left to right).

longer for CT muscles, however. Half-relaxation times averaged 25% longer and 90% relaxation times averaged 43% longer for CT muscles. Only the 90% times were significantly different. With 10 Hz stimulation for 1 s, which gave an unfused tetanus, the differences in relaxation times between CT and C muscles were more pronounced. Half-relaxation times from the tenth twitch averaged 55% longer and 90% relaxation times averaged 93% longer for CT muscles. Both of these differences were significant. Relaxation from a fused tetanus elicited by 50 Hz stimulation for 1 s, was less influenced by testosterone, but was still significantly longer for CT muscles. Half-relaxation times averaged 26% longer and 90% relaxation times averaged 15% longer for CT muscles. Only the half-relaxation times were significantly different. In addition, the half-contraction time of the tetanus (the time to reach 50% of peak tension), was 32% longer for CT muscles, but this difference was not significant.

Testosterone treatment also influenced muscle contractions elicited by stimulation of the FCR nerve (Table 1, bottom section). As with direct stimulation, twitch contraction times did not differ between CT ($n = 10$) and C ($n = 10$) muscles. Twitch relaxation times were, however, significantly longer for CT muscles. Half-relaxation times averaged 57% longer and 90% relaxation times averaged 76% longer for CT muscles. Relaxation from the tenth twitch of a 1 s, 10 Hz stimulus train was also significantly longer for CT muscles. Half-relaxation times averaged 38% longer and 90% relaxation times averaged 50% longer for CT muscles. Relaxation from a fused tetanus elicited by nerve stimulation (50 Hz for 1 s) was not significantly different between CT and C muscles. The half-contraction time of the tetanus, however, was significantly longer for CT muscles (69%).

A comparison of direct *vs.* nerve-stimulated twitches revealed that twitch duration was longer with direct stimulation of CT (Fig. 1*A*) and C (Fig. 1*B*) muscles. For CT muscles, twitches evoked by direct *vs.* nerve stimulation averaged 27% longer for contraction times, 14% longer for half-relaxation times and 5% longer for 90% relaxation times. Only the contraction times were significantly different ($P < 0.001$).

TABLE 3. Contractile strength of FCR muscles

	A Twitch amplitude (N)	B 10 Hz amplitude ratio (10:1)	C Tetanus amplitude (N)
Direct stimulation			
CT	0·22±0·03	1·92±0·13	0·82±0·06
C	0·19±0·03	1·50±0·11*	0·43±0·13**
Nerve stimulation			
CT	0·12±0·02‡	1·93±0·16	0·52±0·07‡
C	0·13±0·01†	1·38±0·06**	0·37±0·06

Values are means±S.E.M. Markers for P values are the same as in Table 1.

For C muscles, twitches evoked by direct *vs.* nerve stimulation averaged 18% longer for contraction times, 43% longer for half-relaxation times and 29% longer for 90% relaxation times. All these differences were significant ($P < 0.01$).

For comparison with experimental muscles, we measured twitches evoked by nerve stimulation for six FCR muscles from three unoperated frogs. These frogs had plasma testosterone levels that averaged 13 ng/ml. Contraction time averaged 18·5±0·5 ms while 50% and 90% relaxation times averaged 13·5±0·4 and 35·0±1·4 ms, respectively. Plasma testosterone levels were within the range seen in our experimental animals, but twitch contraction times were significantly shorter for normal compared to CT or C muscles ($P < 0.01$). Relaxation times of normal muscles were similar to those found for C muscles but significantly faster than CT muscle half-relaxation times ($P < 0.01$) and 90% relaxation times ($P < 0.05$).

Contractions of CR and IL muscles were elicited by nerve stimulation only. Direct stimulation of CR and IL muscles was not attempted in part because the thickness of some muscles made it difficult to recruit all fibres with brief stimulus pulses. The contractile kinetics of CR and IL muscles are summarized in Table 2. The only significant effect of testosterone treatment on CR muscles was a slight slowing (34%) of the half-contraction time for fused tetanic contractions. The contractile kinetics of IL muscles were similarly unaffected by testosterone treatment. The only significant effects observed were a 33% lengthening of the twitch 90% relaxation times and a 36% increase in tetanic contraction times.

Effects of testosterone treatment on the strength of contraction

Testosterone treatment affected the strength of FCR contractions at some, but not every stimulus frequency tested. Average twitch tensions are shown in Table 3A. There was no significant difference between CT and C muscles in directly evoked

twitch tensions or nerve-evoked twitch tensions. Testosterone treatment did influence tension levels generated by repetitive stimuli, however. Contractions elicited with 10 Hz stimuli can be seen in Fig. 2. Summation (measured as the amplitude ratio of the tenth to the first twitch) approximately doubled tension by

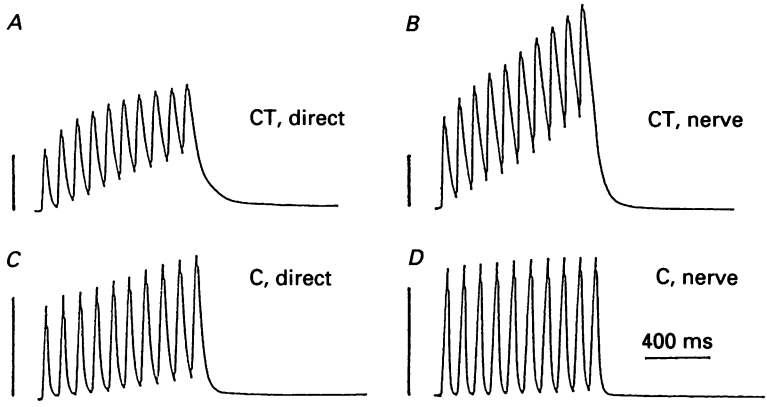

Fig. 2. Summation of tension with 10 Hz stimulation for 1 s. *A*, CT muscle with direct stimulation. *B*, CT muscle with nerve stimulation. *C*, C muscle with direct stimulation. *D*, C muscle with nerve stimulation. Scale bars are 0·2 N for *A* and *C*, 0·1 N for *B* and *D*.

the tenth twitch in CT muscles stimulated directly (Fig. 2*A*) or via the nerve (Fig. 2*B*). In comparison, summation in C muscles was $\leqslant$ 1·5 times for direct (Fig. 2*C*) and nerve (Fig. 2*D*) stimulation. The average summation (amount $>$ 1·0) for CT compared to C muscles (Table 3B) was 84% more with direct stimulation and 144% more with nerve stimulation. These differences were significant. Although tetanic tension was higher for CT compared to C muscles with nerve stimulation, the difference was not significant. Tetanic tension for CT muscles was significantly larger (86%) with direct stimulation of the FCR, however. To determine if this difference in contractile strength was related to size differences between muscles, cross-sectional areas were measured for all the fibres of three CT and three C muscles. The mean cross-sectional area of fibres ($5172 \pm 53\ \mu m^2$, $n = 2657$) from CT muscles was 84% larger than fibres ($2812 \pm 29\ \mu m^2$, $n = 2759$) from C muscles.

Testosterone treatment had little influence on the contractile strength of CR and IL muscles stimulated via the nerve. The data are summarized in Table 4. The only significant difference between CT and C muscles was in the summation of tension in the IL with 10 Hz stimulation (19%).

Effects of testosterone treatment on synaptic efficacy

Tension recordings suggested that nerve stimulation did not recruit all the muscle fibres in the FCR. Twitch amplitudes were always greater with direct *vs.* nerve stimulation of individual muscles (Fig. 1). On average, twitch tension was 83% greater with direct compared to nerve stimulation of CT muscles and 46% greater

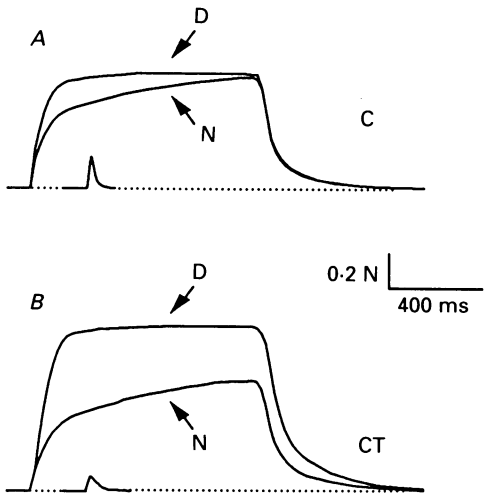

Fig. 3. Comparison of the nerve-evoked twitch, nerve-evoked tetanus (N), and directly evoked tetanus (D) for C (*A*) and CT (*B*) FCR muscles. With nerve stimulation, tetanus tension does not plateau until the final 100 ms of a 1 s stimulation.

TABLE 4. Contractile strength of CR and IL muscles

	A Twitch amplitude (N)	B 10 Hz amplitude ratio (10:1)	C Tetanus amplitude (N)
CR			
CT	0·38±0·03	1·28±0·06	1·27±0·14
C	0·42±0·09	1·26±0·13	1·10±0·13
IL			
CT	0·29±0·03	1·33±0·03	0·86±0·06
C	0·35±0·04	1·12±0·04**	0·77±0·03

Values are means±S.E.M. Markers for P values are the same as in Table 1.

for C muscles (Table 3A). Twitches were also slower with direct muscle stimulation (see above). These results suggested that a single supramaximal stimulus to the nerve failed to recruit a population of fibres with slower twitch kinetics. Presumably these fibres had neuromuscular junctions with low synaptic efficacy (see Discussion).

A comparison of direct and nerve-evoked tetanus tensions revealed that even 50 Hz nerve stimulation did not recruit the entire population of fibres. Figure 3 shows the relationship between the nerve-stimulated twitch, nerve-stimulated tetanus and directly stimulated tetanus for a C muscle (Fig. 3*A*) and a CT muscle (Fig. 3*B*). With nerve stimulation the tetanus often did not plateau until just before the end of the 1 s stimulation. The tetanic tension produced by nerve stimulation is much closer in amplitude to directly stimulated tetanic tension in C muscles compared to CT muscles. When data for six CT and six C muscles were averaged,

tetanus tension was 58% greater with direct compared to nerve stimulation for CT muscles (0·82 *vs.* 0·52 N, $P < 0·01$), but for C muscles the difference (16%) was not significant (0·43 *vs.* 0·37 N).

Differences in synaptic efficacy were also indicated by the ratios of tetanus to twitch amplitudes. Tetanus: twitch ratio is usually expressed using the nerve-evoked

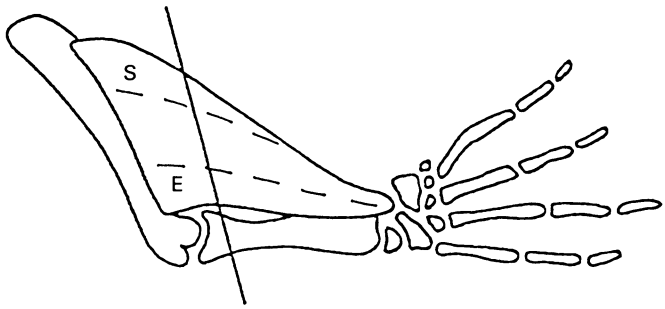

Fig. 4. Drawing of an FCR muscle and associated bones of the forelimb. The dashed lines demarcate regions of fibres used for comparisons. S, shoulder; E, elbow. The diagonal line through the muscle approximates the location of the muscle cross-sections used to measure fibre size.

tetanus and twitch, and is considered roughly proportional to the fraction of fibres innervated by junctions that are subthreshold with single nerve stimuli. Since 50 Hz nerve stimulation did not recruit the entire population of fibres, we also compared directly stimulated tetanic tension to nerve-evoked twitch tension. Twitches were much closer to tetanus in amplitude for muscles of C compared to CT frogs. When data for six CT and six C muscles were averaged, the nerve tetanus:nerve twitch ratio was 76% greater for CT muscles compared to C muscles ($5·8 \pm 0·8$ *vs.* $3·3 \pm 0·2$, $P < 0·01$). The average direct tetanus:nerve twitch ratio was 111% greater for CT muscles compared to C muscles ($7·8 \pm 2·1$ *vs.* $3·7 \pm 0·7$, $P < 0·002$). These results further suggested that testosterone treatment reduced synaptic efficacy.

Synaptic efficacy in CR and IL muscles, measured as the nerve tetanus:nerve twitch ratio, was not as greatly affected by testosterone treatment as in FCR muscles. The average tetanus:twitch ratio was not significantly different between CR muscles from CT ($n = 8$) compared to C ($n = 4$) frogs ($3·3 \pm 0·40$ *vs.* $2·7 \pm 0·23$). The tetanus:twitch ratios of IL muscles, however, were 35% greater in CT ($n = 7$) compared to C ($n = 6$) frogs ($3·1 \pm 0·22$ *vs.* $2·3 \pm 0·21$, $P < 0·05$). These results suggested that testosterone treatment also decreased synaptic efficacy in IL muscles.

Differential effects of testosterone treatment on FCR muscle regions

We have previously shown that testosterone causes differential hypertrophy of FCR fibres (Regnier & Herrera, 1990), with fibres in the shoulder region (S in Fig. 4) growing much more than fibres in the elbow region (E in Fig. 4). This prompted us to examine if the contractile kinetics of shoulder fibres were more affected by testosterone than elbow fibres. Fibres in the shoulder and elbow regions were isolated by cutting muscles into longitudinal strips (dashed lines in Fig. 4). Each strip

TABLE 5. Contractile kinetics of muscle fibre strips and from SN stimulation (ms)

	A Fibre strips				B Spinal nerve stimulation			
	CT		C		CT		C	
	Shoulder	Elbow	Shoulder	Elbow	SN 2	SN 3	SN 2	SN 3
Contraction time	31·0±2·9*	22·0±0·7	26·0±3·0	21·0±1·3	25·0±1·4**	18·0±0·5	22·0±1·0	20·0±1·0
Half-relaxation time	33·0±6·0	21·0±1·9	26·0±4·4	20·0±2·7	22·0±2·2*	16·0±1·1	17·0±2·1	15·0±2·0
90% relaxation time	94·0±11·1*	57·0±6·0	61·0±13·1	47·0±8·7	51·0±7·4	40·0±4·4	45·0±6·1	38·0±5·6

Values are means±S.E.M. Markers for P values are the same as in Table 1.

contained 100–200 fibres. The twitch kinetics of fibre strips are summarized in Table 5A. Shoulder fibre strips from CT muscles ($n = 4$) contracted and relaxed significantly more slowly than elbow fibre strips. Twitch contraction times of shoulder fibres averaged 41% longer than elbow fibres. Half-relaxation times and 90% relaxation times averaged 57 and 65% longer, respectively, in shoulder fibres. The half-relaxation times were not significantly different, but the other kinetic measures were. In C muscles, shoulder fibre strips ($n = 4$) also contracted and relaxed more slowly than elbow fibre strips, but none of these differences was significant.

The amount of fibre stretch profoundly influences contractile kinetics, with contraction and relaxation times becoming longer at longer muscle lengths (Iaizzo & Poppele, 1990). Control measurements showed no significant differences in sarcomere spacing between shoulder ($2{\cdot}50 \pm 0{\cdot}03$ μm) and elbow ($2{\cdot}40 \pm 0{\cdot}04$ μm) fibre strips at muscle lengths that maximized twitch amplitude (L_0). Further measurements showed that twitch contraction and relaxation times for shoulder and elbow fibre strips did not overlap at muscle lengths between 90 and 110% of L_0. We therefore concluded that differences in kinetics were probably not due to differential stretching of fibres.

An estimate of specific tension was obtained for four CT and three C shoulder fibre strips by normalizing tetanic tension (50 Hz stimulation) for strip cross-sectional area. Specific tension was slightly, but not significantly, greater for CT compared to C shoulder fibre strips ($22{\cdot}5 \pm 3{\cdot}9$ *vs.* $18{\cdot}6 \pm 1{\cdot}0$ N/cm^2). These findings were consistent with whole-muscle measurements of tension and muscle size (see above), suggesting that differences in tetanic tension between CT and C muscles were due largely to differences in fibre size.

FCR motor units are differentially affected by testosterone

The FCR muscle is innervated by motor axons in spinal nerves 2 and 3 (SN 2 and SN 3). During the course of experiments it was noticed that stimulation of SN 2 elicited slower muscle contractions than stimulation of SN 3. A summary of twitch kinetics for separate spinal nerve stimulations of muscles is given in Table 5B. Contraction times of CT muscles ($n = 6$) were 39% longer with SN 2 stimulation than with SN 3 stimulation, a significant difference. Half- and 90% relaxation times were also longer with SN 2 stimulation but only the half-relaxation times were significantly different. For C muscles ($n = 6$), twitches evoked by SN 2 and SN 3 stimulation were not significantly different in contraction or relaxation times.

Visual inspection of contracting muscles revealed that SN 2 mainly innervates the shoulder region of the FCR and SN 3 mainly innervates the remainder of the muscle. This pattern has been confirmed by intracellular recording (Nagaya & Herrera, 1991). It appears, therefore, that motor axons in SN 2 innervate the region of the FCR that is most sensitive to testosterone.

DISCUSSION

Results of the present study indicate that testosterone enhances the ability of FCR muscles in males to produce and maintain tension. These changes are seemingly due to a differential effect of testosterone on fibres in the shoulder region of the muscle.

Presumably the control of fibre properties by testosterone is at the motor unit level, since fibres innervated by axons in spinal nerve 2 are affected more than fibres innervated by axons in spinal nerve 3. Differential sensitivity to testosterone may also occur in FCR motoneurones. Erulkar *et al.* (1981) found that motoneurones in the brachial enlargement that accumulate dihydrotestosterone are predominantly concentrated in the second spinal segment.

Contractile kinetics of the FCR

With direct stimulation, contraction times of FCR muscles (22–31 ms) were within the range of times reported for the 'fast' sartorius muscle in *Rana temporaria* (27·5 ± 0·73 ms, 20 °C, Thibert & Nicolet, 1975) and *Litoria aurea* (21–36 ms, 22–24 °C, Luff & Proske, 1976). Contraction times of FCR muscles were also similar to those we obtained for IL muscles (23–34 ms). The *Xenopus* IL muscle is heterogeneous in composition. Lännergren & Smith (1966) found that twitch contraction times for single IL fibres varied over a fourfold range (20–80 ms). Like the IL, the histochemical profile of the FCR is diverse (Hayatsu, Kosaka & Hasumi, 1978; Oka, Ohtani, Satou & Ueda, 1984; Thibert, 1986; M. Regnier & A. A. Herrera, unpublished observations) and single fibre contraction times probably vary over a wide range. Twitch contraction times of shoulder fibre strips are within ranges reported for the fastest two fibre types proposed by Lännergren and his co-workers (Lännergren & Smith, 1966; Lännergren, Lindblom & Johansson, 1982).

There is evidence that androgens influence contractile kinetics in other muscles. Twitch duration of FCR muscles of *Rana temporaria* is shortest during the summer when androgen levels are lowest, lengthens as androgen levels rise in the autumn, and is longest during the breeding season when androgen levels are high (Melichna *et al.* 1972). This increase in twitch duration is due to a lengthening of both contraction and relaxation times. In contrast, castration has been shown to cause a prolongation of contraction and relaxation times in the levator ani of rats, an effect that can be reversed with testosterone treatment (Vyskocil & Gutmann, 1977). Sassoon, Gray & Kelley (1987) suggest that high androgen levels during postmetamorphic development make male *Xenopus* laryngeal muscle fibres 'faster' than their female counterparts.

There are several possible mechanisms by which testosterone could slow contractions of shoulder region fibres. One possibility is that testosterone influences the myosin isoform composition of muscle fibres. Lyons, Kelley & Rubinstein (1986) demonstrated differences in myosin isozymes between temporalis muscles of male and female guinea-pigs. These differences were eliminated by castration of males or testosterone treatment of females.

Testosterone may cause changes in the sarcoplasmic reticulum that would lengthen isometric contractions. Increases in the duration of isometric contractions during adaptation to chronic electrical stimulation are correlated with decreased Ca^{2+} uptake activity of the sarcoplasmic reticulum but not with myosin ATPase activity (reviewed by Pette, 1984). Little is known about the effects of androgens on the sarcoplasmic reticulum, however. Saborido, Fulgencio & Megias (1991) found that androgens approximately doubled sarcotubular fractions of soleus muscles from sedentary rats, but had no effect on Ca^{2+}-ATPase activity. It would be interesting to

know if testosterone-induced changes in fibre size are accompanied by changes in the amount of sarcoplasmic reticulum or the capacity for Ca^{2+} sequestration.

The slowing of muscle contractions could be secondary to an increase in neural activity. Increasing activity by repetitive, low-frequency stimulation of 'fast' mammalian muscles *in vivo* slows contraction kinetics (Salmons & Streter, 1976; reviewed by Pette & Vrbova, 1985). This activity-induced slowing of contractile kinetics is correlated with changes to 'slower' myosin isoforms (Barany & Close, 1971; reviewed by Pette, 1984) and a decreased sarcoplasmic reticulum volume (Heilmann, Muller & Pette, 1981; Klug, Wicker, Reichmann, Leberer & Pette, 1983). Erulkar *et al.* (1981) demonstrated an androgen-induced change in the electrical behaviour of the *Xenopus* spinal cord that could contribute to increased motoneuronal activity. Administration of dihydrotestosterone to the *in vitro* spinal cord increased the response of CR motoneurones to dorsal root stimulation.

Contractile strength of FCR muscles

Differences in tetanic contractile strength between CT and C FCR muscles (86%) were similar to differences in average fibre size (84%). These findings suggest that testosterone treatment did not affect specific tension in FCR muscles. Estimates of specific tension measured for the shoulder region also suggest that specific tension was not greatly affected. The average specific tension of shoulder fibre strips from CT and C muscles was not significantly different. Our estimates of specific tension should be considered as low estimates because 50 Hz stimulation did not elicit maximal tetanic tension in FCR muscles. Measurements from control muscles revealed that tension levels reached maximum at 110–130 Hz stimulation. Nevertheless, the average specific tension of shoulder fibres (22 N/cm^2) was similar to values obtained for fast motor units in mammalian muscles (22–34 N/cm^2; reviewed by Burke, 1981).

Synaptic efficacy of FCR muscles

Neuromuscular junctions in frog skeletal muscles normally have transmitter release levels close to the minimum necessary to depolarize fibres to action potential threshold. Indeed, when nerves are stimulated at low frequencies *in vitro*, many junctions are subthreshold (reviewed in Grinnell & Herrera, 1981). In FCR muscles, the observation that twitch contractions are slower with direct compared to nerve stimulation suggests that, on average, neuromuscular junctions of slower fibres have lower synaptic efficacy. In addition, two lines of evidence suggest that the number of low-efficacy junctions increases when testosterone levels are high. First, direct tetanus:nerve twitch ratios averaged over twofold greater in muscles from CT compared to C frogs, indicating that average synaptic efficacy may be decreased by testosterone treatment. The second line of evidence is that nerve stimulation produced only about half the tension of direct stimulation in CT muscles (see Table 3), indicating a large population of subthreshold junctions. The difference in tension produced by nerve *vs.* direct stimulation of C muscles is much less, indicating fewer subthreshold junctions.

There is the possibility that differences in tension produced by nerve *vs.* direct stimulation of the same muscle could be due to damage of some axons innervating the muscle. There is, however, substantial evidence to suggest that this is not the

case. First, the ratio of tension for nerve:direct stimulation was always lower in CT muscles than in C muscles. It is unlikely that axonal damage was consistently greater in CT muscles. Second, Nagaya & Herrera (1991) have shown, using intracellular recording, that only 63% of shoulder neuromuscular junctions in CT muscles triggered muscle fibre action potentials with a single nerve stimulus compared to 97% of shoulder junctions in C muscles. These results support our conclusion that lower tension production in CT muscles with nerve stimulation *vs.* direct stimulation is most likely due to lower synaptic efficacy in CT muscles.

Differences in synaptic efficacy between CT and C muscles could be due, at least partially, to differences in fibre input resistance. Muscle fibre input resistance varies inversely with fibre size, and shoulder fibre size is very dependent on testosterone (Regnier & Herrera, 1991). Nagaya & Herrera (1991) have found that input resistance in shoulder fibres of CT muscles averages only 66% of that found in the same fibres in C muscles. It is possible that prolonged exposure to high androgen levels (8 weeks) resulted in fibre hypertrophy beyond that seen normally during breeding. Transmitter release may not have increased enough to compensate for the decreased input resistance in shoulder fibres, thereby diminishing synaptic efficacy.

There is some evidence that androgens can affect motoneurones and neuromuscular junctions in other sexually dimorphic systems (reviewed in Herrera & Regnier, 1991). Vyskocil & Gutmann found that castration caused no significant short term (1969) or long term (1977) changes in the frequency of spontaneous miniature endplate potentials (MEPPs) in levator ani muscles of rats. Administration of testosterone to castrates for seven days, however, increased MEPP frequency by 92%. In *Xenopus* laryngeal muscles, sexual dimorphisms in synaptic function have been described (Tobias & Kelley, 1988) as well as effects of androgens on the opening of acetylcholine receptor channels (Erulkar & Wetzel, 1989).

Contractile properties of the CR and IL muscles

Testosterone treatment had very little effect on the contractile properties of CR and IL muscles. Only nerve-stimulated contractions were measured in these muscles, however. Changes in contraction strength would be best measured with direct muscle stimulation since synaptic efficacy can greatly influence fibre recruitment. The CR, sometimes called the sternoradialis, is a sexually dimorphic forelimb flexor. In males, CR muscles are larger than in females (Kirby, 1983; Oka *et al.* 1984). Fibre number and histochemical staining profiles are similar (Oka *et al.* 1984), however, indicating the CR may be less dimorphic than other forelimb flexors. There is little evidence that the CR is androgen sensitive in adult males. Thibert & Nicolet (1972) found no difference between normal and castrated male *Rana temporaria* in tetanic tension produced by direct muscle stimulation. Oka *et al.* (1984) found no seasonal differences in histochemical staining for ATPase or succinate dehydrogenase in male *Bufo japonicus*, in spite of seasonal fluctuations in testosterone levels. We now report that nerve-evoked twitch and tetanus tension are unaffected by testosterone levels in the CR of male *Xenopus*.

Almost nothing is known about whether hindlimb muscles in frogs are sexually dimorphic or androgen sensitive. Segil, Silverman & Kelley (1987) reported that a male thigh muscle (gluteus) has androgen binding levels 16 times lower than the androgen-sensitive larynx. It is not known whether all thigh muscles have similarly

low capacities for androgen binding. To our knowledge, no-one has reported sexual dimorphism or androgen sensitivity for the IL muscle. Our findings suggest there is some sensitivity to testosterone. Relaxation times for nerve-evoked twitches were slightly longer for CT compared to C muscles. Longer relaxation times resulted in slightly greater summation of tension with 10 Hz stimulation. Testosterone treatment also caused a decrease in synaptic efficacy, as evidenced by tetanus:twitch ratios.

Functional consequences

Clasping behaviour has been observed in most, if not all, anuran species but is particularly well described for *Xenopus laevis* (Russell, 1954, 1960; Hutchison & Poynton, 1963; Kelley & Pfaff, 1976). These studies show that the forelimbs of clasping males must generate two different patterns of flexion: (1) a prolonged but loose maintenance grip and (2) brief, rapid tightening. The present results show that under the influence of testosterone, the FCR muscle becomes well adapted to meet these functional demands. Twitch relaxation is prolonged, which makes it possible to maintain minimal levels of tension with less neuromuscular activity. At the same time, the FCR retains its ability to contract rapidly and with greater than normal force.

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