NONGENOMIC ACTIONS OF PROGESTERONE AND 17β-ESTRADIOL ON THE CHLORIDE CONDUCTANCE OF SKELETAL MUSCLE

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ABSTRACT: *Introduction*: Myotonia congenita, caused by mutations in *ClC-1*, tends to be more severe in men and is often exacerbated by pregnancy. *Methods*: We performed whole-cell patch clamp of mouse muscle chloride currents in the absence/presence of 100 μM progesterone or 17β-estradiol. *Results*: 100 μM progesterone rapidly and reversibly shifted the ClC-1 activation curve of mouse skeletal muscle (V50 changed from -52.6 ± 9.3 to $+35.5 \pm 6.7$; $P < 0.01$) and markedly reduced chloride currents at depolarized potentials. 17β-estradiol at the same concentration had a similar but smaller effect (V50 change from -57.2 ± 7.6 to -40.5 ± 9.8; $P < 0.05$). 1 μM progesterone produced no significant effect. *Conclusions*: Although the data support the existence of a nongenomic mechanism in mammalian skeletal muscle through which sex hormones at high concentration can rapidly modulate ClC-1, the influence of hormones on muscle excitability *in vivo* remains an open question.

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The voltage-gated chloride channel, ClC-1, is important in regulation of muscle excitability; it matches the properties of a myofiber with the demands of its motor neuron[1] and combats fatigue.[2,3] A role for sex hormones in muscle excitability is suggested by the observations that the chloride channel disease myotonia congenita tends to be more severe in men and is often exacerbated by pregnancy.[4,5] In *Xenopus* oocytes, ClC-1 can be modulated by sex hormones through a rapid, and, therefore, nongenomic, mechanism.[7] We asked whether such a mechanism exists in mammalian skeletal muscle.

MATERIALS AND METHODS

Isolated flexor digitorum brevis (FDB) myofibers from mice aged between 1 and 4 months were studied by whole-cell patch clamp following the method of Lueck et al.[6] Briefly, animals were killed by isoflurane inhalation and cervical dislocation, and the FDB muscle from a hindfoot was triturated in a series of 35-mm plastic tissue culture dishes of Ringer solution (in mM: NaCl 146, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10 [(4-(2-hydroxyethyl)−1-piperazineethanesulfonic acid], pH 7.4) after incubation for 1 h at 37°C in 1 mg/ml collagenase A dissolved in Ringer solution. Animals were shared with a colleague conducting experiments on brain, some of which harbored a mutation in *Kv1.1*. In the experiments presented here, Kv channels were blocked by tetraethylammonium (TEA). No differences were observed between mutant and wildtype animals with respect to the hormone sensitivity of chloride conductance.

Data were collected using fire-polished thin glass microelectrodes with an Axopatch 200B, sampling at 10 kHz with a 2 kHz low-pass filter. Series resistance was 1–3 MΩ and was compensated 90%. Recording solutions at pH 7.4 contained in mM, internal: Cs-Aspartate 110, Cs-Cl 30, $MgCl_2$ 5, HEPES 10; external: TEA-Cl 145, $CaCl_2$ 10, $CdCl_2$ 0.25, HEPES 10. Ringer and external solutions was supplemented with 10 μM BTS to reduce contractions. Stock solutions of BTS, progesterone, and 17β-estradiol were made in dimethyl sulfoxide (DMSO). The total concentration of DMSO in the extracellular recording solution did not exceed 0.22%.

Tail currents were measured without leak subtraction at −100 mV after pre-pulses to voltages from −140 to +120 mV. For each cell, the maximal prehormone current was used to normalize both the pre- and posthormone currents.

Pre- and posthormone parameters for progesterone and estrogen were compared in paired *t*-tests. The effect of progesterone alone, estrogen alone, and progesterone after prior exposure to estrogen were compared in a one-way analysis of variance.

RESULTS

In the presence of 100 μM progesterone the ClC-1 activation curve was shifted in a depolarizing direction (V50 changed from -52.6 ± 9.3 mV to $+35.5 \pm 6.7$ mV, $P < 0.01$), and peak current was reduced with the overall effect of reducing chloride currents at depolarized potentials (Fig. 1).

Abbreviations: DMSO, dimethyl sulfoxide; FDB, flexor digitorum brevis; HEPES, 4-(2-hydroxyethyl)−1-piperazineethanesulfonic acid; TEA, tetraethylammonium

Key words: chloride channel; excitability; myotonia congenita; sex hormones; skeletal muscle

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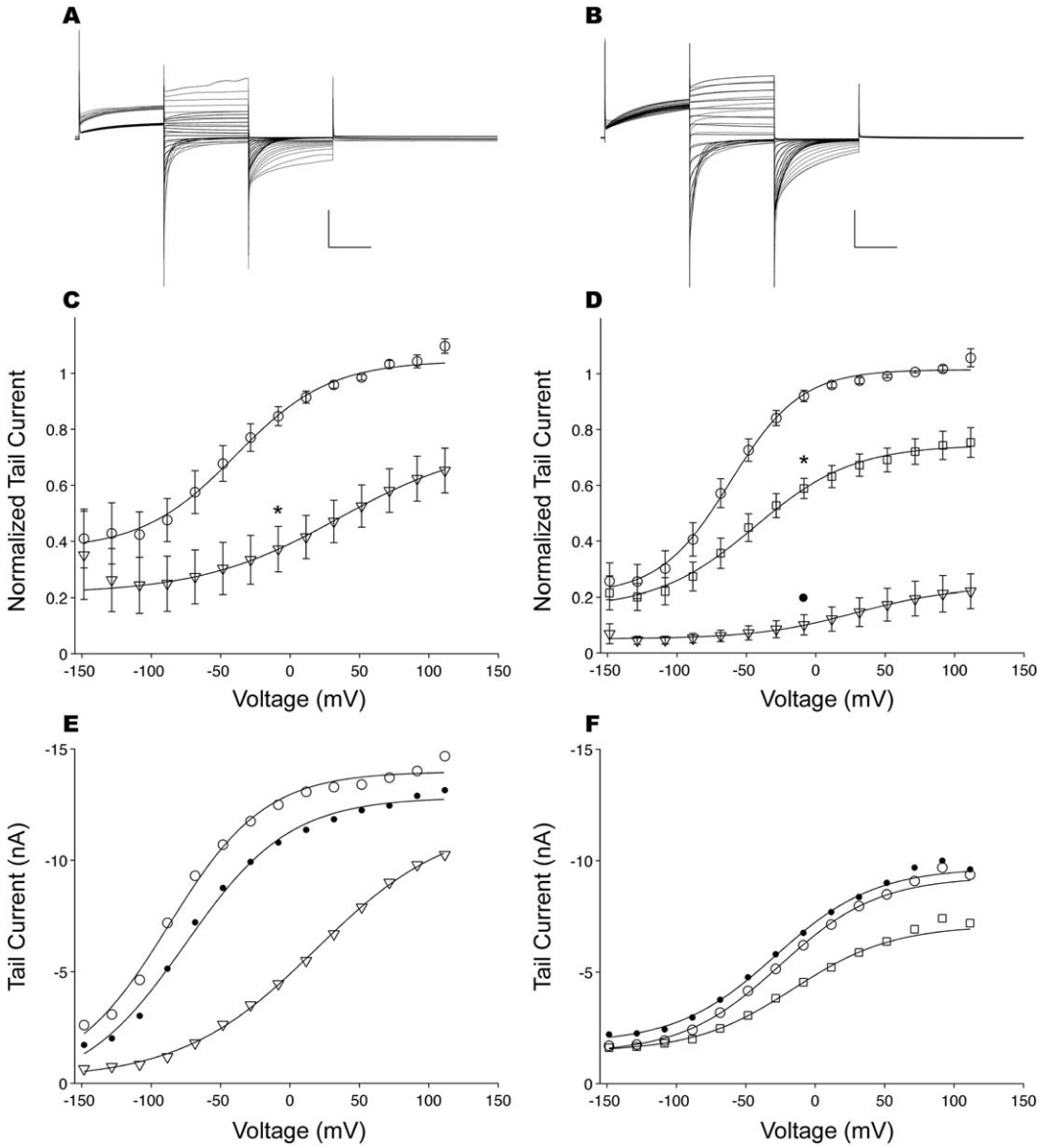

FIGURE 1. Comparison of chloride tail currents before and after application of 100 μM 17β-estradiol (left) or progesterone (right) to the bath. Top row: Representative chloride currents before (grey) and after (black) 100 μM hormone. (**A**) A cell before/after progesterone. (**B**) A different cell before/after 17β-estradiol. Scale bars show 200 ms horizontally, 20 nA vertically. Middle row: Fraction tail current as a proportion of pre-hormone maximal current. Means ± SEM are displayed with Boltzmann fits. Circles in C and D are before application of hormone. (**C**) The effect of 100 μM progesterone (triangles, n = 5). (**D**) The 17β-estradiol was applied first (squares, n = 5) and after washoff, progesterone was applied (triangles; n = 3). Tail currents at 0 mV were significantly smaller in the presence of progesterone or 17β-estradiol (stars, $P < 0.01$, paired *t*-tests). Progesterone after prior exposure to 17β-estradiol produced significantly smaller tail currents at 0 mV than either progesterone or 17β-estradiol alone (dot, $P < 0.01$, one-way analysis of variance and post hoc pairwise analysis). Bottom row: Effects of progesterone (**E**) and 17β-estradiol (**F**) are reversed by washing hormone off. Before hormone (open circles), after 100 μM hormone (triangles, squares), wash (closed circles), n = 1.

The 17β-estradiol at the same concentration had a similar but smaller effect (V50 change from -57.2 ± 7.6 mV to -40.5 ± 9.8 mV, $P < 0.05$). These effects were complete within the time it took to exchange the solutions and re-start recording (minutes) and could be reversed by washing the hormone off with a similar time scale, suggesting a nongenomic mechanism. However, 1 μM progesterone produced no significant effect (data not shown).

DISCUSSION

In *Xenopus* oocytes sex hormones rapidly reduce the conductance through heterologously expressed human ClC-1[7]; we found that the endogenous chloride conductance in mammalian skeletal muscle responds similarly. The components of the pathway linking sex hormones and ClC-1 remain to be explored. Protein kinase C is known both to regulate ClC-1[8,9] and to mediate certain nongenomic actions of progesterone in

the brain.[10] However, as in the oocyte experiments, the concentration of hormone that was effective in our recordings is much higher than what occurs naturally in blood; serum progesterone levels during late pregnancy are approximately 0.2 μM.[11] Thus, although the data support the existence of a mechanism in skeletal muscle through which sex hormones at high concentration can rapidly modulate ClC-1, the influence of hormones on muscle excitability *in vivo* remains an open question.

REFERENCES

1. Klocke R, Steinmeyer K, Jentsch T, Jockusch H. Role of innervation, excitability and myogenic factors in the expression of the muscular chloride channel ClC-1. J Biol Chem 1994;269:27635–27639.
2. Pedersen TH, Nielsen OB, Lamb GD, Stephenson DG. Intracellular acidosis enhances the excitability of working muscle. Science 2004; 305:1144–1147.
3. de Paoli FV, Ortenblad N, Pedersen TH, Jorgensen R, Nielsen OB. Lactate per se improves the excitability of depolarized rat skeletal muscle by reducing the Cl- conductance. J Physiol 2010;588(Pt 23):4785–4794.
4. Colding-Jorgensen E. Phenotypic variability in myotonia congenita. Muscle Nerve 2005;32:19–34.
5. Fialho D, Schorge S, Pucovska U, Davies NP, Labrum R, Haworth A, et al. Chloride channel myotonia: exon 8 hot-spot for dominant-negative interactions. Brain 2007;130(Pt 12):3265–3274.
6. Lueck JD, Mankodi A, Swanson MS, Thornton CA, Dirksen RT. Muscle chloride channel dysfunction in two mouse models of myotonic dystrophy. J Gen Physiol 2007;129:79–94.
7. Fialho D, Kullmann DM, Hanna MG, Schorge S. Non-genomic effects of sex hormones on CLC-1 may contribute to gender differences in myotonia congenita. Neuromuscul Disord 2008;18:869–872.
8. Brinkmeier H, Jockusch H. Activators of protein kinase C induce myotonia by lowering chloride conductance in muscle. Biochem Biophys Res Commun 1987;148:1383–1389.
9. Rosenbohm A, Rudel R, Fahlke C. Regulation of the human skeletal muscle chloride channel hClC-1 by protein kinase C. J Physiol 1999; 514(Pt 3):677–685.
10. Balasubramanian B, Portillo W, Reyna A, Chen JZ, Moore AN, Dash PK, et al. Nonclassical mechanisms of progesterone action in the brain: I. Protein kinase C activation in the hypothalamus of female rats. Endocrinology 2008;149:5509–5517.
11. Soldin OP, Guo T, Weiderpass E, Tractenberg RE, Hilakivi-Clarke L, Soldin SJ. Steroid hormone levels in pregnancy and 1 year postpartum using isotope dilution tandem mass spectrometry. Fertil Steril 2005;84:701–710.

LOCAL ANESTHETIC EFFECTS ON GENE TRANSCRIPTION IN HUMAN SKELETAL MUSCLE BIOPSIES

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ABSTRACT: *Introduction:* We examined if epinephrine in the local anesthetic to help control incision-related bleeding interferes with molecular measurements obtained with the Duchenne-Bergström percutaneous needle biopsy technique for sampling human skeletal muscle. *Methods:* Three groups received 2.5–3.0 ml of 1% lidocaine in 2 injections: (1) 0.5–1.0 ml superficially, which varied among the groups according to (i) −Epi; intra- and subcutaneous without epinephrine, (ii) +Epi −Fascia; intra- and subcutaneous with epinephrine, avoiding the fascia, and (iii) +Epi +Fascia; intra- and subcutaneous with epinephrine, directing a small amount (∽0.2 ml) into the fascia area; and (2) ∽2.0 ml without epinephrine into the fascia area for all subjects. A muscle biopsy was obtained 5–10 min later for IL-6 and MuRF-1 mRNA levels. *Results*: IL-6 mRNA levels were low in −Epi and +Epi −Fascia, but ∽300-fold higher in +Epi +Fascia. MuRF-1 mRNA levels were similar among the groups. *Conclusions*: Lidocaine with epinephrine can confound intramuscular measurements from needle biopsies, but this can be avoided with a careful injection approach.

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Local anesthetic supplemented with epinephrine has been used for the Duchenne–Bergström percutaneous needle muscle biopsy technique[1,2] in human exercise physiology and clinical investigations for at least 40 years.[3,4] The epinephrine helps reduce bleeding associated with the incision in the skin, subcutaneous tissue, and fascia. Although we have excluded use of epinephrine in our muscle biopsy approach for the last ∽15 years in order to eliminate any possible effects on potentially sensitive molecular events and regulatory pathways, we recently reintroduced it in a limited way for its original vasoconstrictive effects. As a result of introducing a small amount of lidocaine with epinephrine (<1.0 ml, 1:100,000) only superficially, but including the muscle fascia area, we discovered unexpectedly that intramuscular transcription can be extremely sensitive to this approach. These results were compared with a modified injection approach to delivering the lidocaine with epinephrine component and with not having any epinephrine in the local anesthetic injection.

METHODS

Fifteen healthy, moderately active men (age 24 ± 1 years, height 182 ± 2 cm, weight 82.0 ± 2.5 kg) were studied as part of ongoing investigations at the Human Performance Laboratory, which were approved by the institutional review board of Ball State University. All subjects provided written consent to participate. Subjects underwent a muscle biopsy of the vastus lateralis muscle with a 5-mm

Abbreviations: ANOVA, analysis of variance; Epi, epinephrine; IL-6, interleukin-6; MuRF-1, muscle RING finger-1

Key words: epinephrine; IL-6; lidocaine; MuRF-1; xylocaine

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