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CHAPTER 2B

ANDROGENIC SUPPRESSION OF SPREADING DEPRESSION IN FAMILIAL HEMIPLEGIC MIGRAINE TYPE 1 MUTANT MICE

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ABSTRACT

Familial hemiplegic migraine type 1 (FHM1), a severe migraine with aura variant, is caused by mutations in the *CACNA1A* gene. Mutant mice carrying the FHM1 R192Q mutation exhibit increased propensity for cortical spreading depression (CSD), a propagating wave of neuroglial depolarization implicated in migraine aura. The CSD phenotype is stronger in female R192Q mutants and diminishes after ovariectomy. Here, we show that orchiectomy reciprocally increases CSD susceptibility in R192Q mutant mice. Chronic testosterone replacement restores CSD susceptibility by an androgen receptor-dependent mechanism. Hence, androgens modulate genetically-enhanced CSD susceptibility and may provide a novel prophylactic target for migraine.

INTRODUCTION

Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with aura associated with transient hemiparesis. Aura and headache features are otherwise identical to those in common forms of migraine.¹ FHM1 is caused by missense mutations in the *CACNA1A* gene, which encodes the pore-forming α_{1A} -subunit of neuronal $\text{Ca}_v2.1$ voltage-gated Ca^{2+} channels (VGCC).² When expressed in transfected cultured neurons, FHM1 mutations shift channel opening toward more negative membrane potentials and delay channel inactivation. Channels open with smaller depolarization and stay open longer, allowing more Ca^{2+} to enter presynaptic terminals.^{3,4} Increased action potential-evoked Ca^{2+} influx has been shown to enhance excitatory neurotransmission at pyramidal cell synapses of FHM1 mutant mice.⁵ Accordingly, mutant mice carrying the FHM1 R192Q mutation show enhanced susceptibility to cortical spreading depression (CSD), the electrophysiological correlate of migraine aura, and a possible trigger of migraine headache mechanisms.^{4,6-8} CSD is characterized by an intense depolarization of neuronal and glial membranes propagating at a rate of ~3mm/minute. Evoked when extracellular K^+ concentrations exceed a critical threshold, CSD is associated with massive K^+ and glutamate efflux, depolarizing adjacent neurons and glia and facilitate CSD spread.

Gonadal hormones are important modulators of migraine and cortical excitability.^{9,10} Incidence of common types of migraine both with or without aura is three-fold higher in females (25%) than in males (8%).¹¹ A female preponderance has also been described for familial (5:2) and sporadic (4.25:1) hemiplegic migraine.^{1,12}

Brennan et al.¹³ recently reported that KCl and electrical stimulation thresholds for CSD induction are both reduced by approximately 50% in wild-type (WT) female mice compared to males. We found a similar increase in CSD susceptibility in female FHM1 knockin mice compared to males; the sex difference was abrogated by ovariectomy and partly restored by estradiol replacement, suggesting that estrogens modulate CSD susceptibility.⁷ Although the female preponderance of migraine has been largely attributed to ovarian sex steroids, anecdotal evidence suggests a role for testosterone and its synthetic derivatives in suppressing migraine in both men and women.¹⁴⁻¹⁶ Here, we provide in vivo experimental evidence for androgenic suppression of CSD susceptibility, as a surrogate model for migraine aura. The data suggest that male and female gonadal hormones exert reciprocal effects on CSD susceptibility, and that androgens may contribute to the lower prevalence of FHM and common types of migraine in males.

MATERIALS AND METHODS

Experimental groups and the number of mice in each group are shown in the Table (n = 106). Adult (4-8 months) or senescent (11-13 months) male FHM1 knockin mice, homozygous for the R192Q mutation that was introduced in the mouse *Cacna1a* gene

by a gene-targeting approach,⁴ were compared to WT littermates and C57BL6/J mice. All experiments were carried out with the investigator blinded for the genotype, and confirmatory genotyping was done after the experiment.

Experiments were conducted in accordance with the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and were approved by the institutional review committee. The femoral artery was catheterized for blood sampling and measurement of mean arterial pressure, and the trachea was intubated for mechanical ventilation under isoflurane anesthesia (2.5% induction, 1% maintenance, in 70% N₂O/30% O₂). Arterial blood gases and pH were measured every 20 minutes and maintained within normal limits by adjusting ventilation (Table). Mice were placed in a stereotaxic frame and burr holes were drilled at the coordinates described previously.⁷ Two glass capillary microelectrodes were placed to record extracellular steady (DC) potential and electrocorticogram at a depth of 300 μ m. After surgical preparation, the occipital cortex was allowed to recover for 20 minutes under saline irrigation. The frequency of CSDs evoked by epidural KCl application (300mM for 30 minutes) was determined, as previously described.⁷ The propagation speed, amplitude, and duration of the first CSD were also measured.

Orchiectomy was performed under brief isoflurane anesthesia 3 weeks prior to CSD susceptibility testing. Subcutaneous testosterone pellets (0.1 mg/pellet, 21-day release; Innovative Research of America, Sarasota, FL) were implanted into the dorsal neck and shoulder region on the day of orchiectomy. The pellets restore physiological circulating levels of testosterone for at least 21 days, but may cause an early peak in plasma levels during the first week after implantation. In order to test whether testosterone replacement exerts its effects on CSD via androgen receptors, a subgroup of orchiectomized testosterone-replaced mice also received pellets containing the androgen receptor antagonist, flutamide (25 or 50 mg/pellet, 21-day release; Innovative Research of America). In addition, acute effects of testosterone propionate (1.2 mg per mouse in 0.1 ml of β -cyclodextrin injected subcutaneously; Sigma, St. Louis, MO) were tested 1 hour before electrophysiological recording in castrated mice. The effectiveness of orchiectomy, testosterone, and flutamide treatments was confirmed by measuring the prostate and seminal vesicle weights after sacrifice.

Data were analyzed using SPSS (version 11.0; SPSS, Inc., Chicago, IL). Using a general linear model of covariance analysis (ANACOVA), we tested for an effect of the independent variables genotype, age, orchiectomy, testosterone treatment (acute, chronic) and flutamide treatment (25mg, 50mg) on the dependent variables CSD frequency and propagation speed. Other electrophysiological measures of CSD and systemic physiological data were compared among groups using one-way analysis of variance (ANOVA). Data are presented as mean $\pm$ standard deviation (SD), and $p < 0.05$ was considered statistically significant.

RESULTS

Continuous epidural KCl application evoked repetitive CSDs in all mice (Figure). Both the frequency and the propagation speed of CSDs were significantly higher in R192Q mutants compared to the WT, as reported previously.⁷ Orchiectomy further increased CSD frequency (by 40%) and to a lesser extent the propagation speed in R192Q mutants, but not in WT mice. Chronic testosterone replacement for 21 days completely prevented the orchiectomy-induced increase in CSD susceptibility in R192Q mutant mice (Figure; Table). In contrast, a single dose of testosterone propionate administered 1 hour before electrophysiological recordings had no effect (16 ± 2 CSDs/hour, 4.3 ± 0.3 mm/minute; $p > 0.05$ vs. orchiectomized controls). The CSD suppression by chronic testosterone replacement was prevented by cotreatment with androgen receptor antagonist flutamide (50 mg pellet); a lower dose of flutamide was ineffective (25 mg pellet; data not shown). Aging (11-13 months) had no effect on CSD frequency and propagation speed in either WT or R192Q mutant mice, consistent with the maintenance of plasma testosterone levels during aging in this WT background strain.¹⁷ In WT mice, gonadectomy or testosterone replacement did not significantly alter CSD susceptibility, suggesting that in our model androgens modulate CSD susceptibility only if the latter is genetically enhanced. The CSD duration and amplitude, and systemic physiological parameters, did not significantly differ among groups (Table).

	n	Age (months)	BW (g)	CSD				Systemic Physiology			
				Frequency (CSD/ hour)	Speed (mm/ minute)	Duration (sec)	Amplitude (mV)	BP (mmHg)	pH _a	p _a CO ₂ (mmHg)	p _a O ₂ (mmHg)
WT											
Naive	10	5 ± 1	30 ± 4	9 ± 1	2.7 ± 0.1	43 ± 11	24 ± 5	81 ± 9	7.37 ± 0.06	35 ± 6	149 ± 28
Orx	8	5 ± 1	27 ± 3	10 ± 1	2.9 ± 0.1	36 ± 9	28 ± 4	83 ± 5	7.36 ± 0.05	35 ± 5	133 ± 19
Orx+T _{Chronic}	4	4 ± 0	27 ± 1	9 ± 1	2.8 ± 0.2	41 ± 7	23 ± 1	86 ± 1	7.40 ± 0.03	31 ± 3	142 ± 18
Aged	5	11 ± 0	37 ± 3	9 ± 1	2.7 ± 0.0	23 ± 9	24 ± 4	87 ± 5	7.43 ± 0.02	31 ± 3	157 ± 19
R192Q											
Naive	19	5 ± 1	27 ± 3	12 ± 1	3.8 ± 0.2	37 ± 10	23 ± 5	84 ± 5	7.38 ± 0.05	32 ± 6	133 ± 22
Orx	16	5 ± 1	27 ± 2	17 ± 2	4.1 ± 0.1	32 ± 8	25 ± 7	86 ± 5	7.37 ± 0.05	32 ± 4	140 ± 20
Orx+T _{Chronic}	16	6 ± 1	28 ± 2	12 ± 1	3.5 ± 0.3	38 ± 9	23 ± 3	88 ± 3	7.38 ± 0.03	30 ± 4	153 ± 22
Orx+T _{Chronic} +F ₂₅	7	8 ± 0	30 ± 6	12 ± 0	3.7 ± 0.2	32 ± 6	18 ± 2	85 ± 7	7.42 ± 0.04	33 ± 5	161 ± 18
Orx+T _{Chronic} +F ₅₀	8	5 ± 0	27 ± 1	17 ± 1	4.2 ± 0.4	33 ± 6	25 ± 3	91 ± 5	7.40 ± 0.06	31 ± 4	157 ± 36
Orx+T _{Acute}	6	7 ± 0	28 ± 2	16 ± 2	4.3 ± 0.3	33 ± 10	25 ± 4	90 ± 5	7.40 ± 0.03	31 ± 3	151 ± 37
Aged	7	13 ± 2	37 ± 4	12 ± 1	3.6 ± 0.3	42 ± 12	27 ± 3	80 ± 6	7.37 ± 0.05	34 ± 5	146 ± 19

Table. Electrophysiological Measures of CSD, and Systemic Physiological Parameters. Values are mean ± SD. CSD duration was measured at one-half amplitude. The duration and the amplitude of only the first CSD are shown. Systemic physiological parameters were averaged over 1 hour recording duration. CSD = cortical spreading depression; SD = standard deviation; BW = body weight; Orx = orchiectomized mice; T_{Chronic} = testosterone 0.1mg pellet for 21 days; T_{Acute} = single 1.2mg dose of testosterone administered 1 hour prior to CSD testing; F_{25/50} = flutamide 25 or 50mg/pellet for 21 days; BP = mean arterial blood pressure; pH_a = acidity of arterial blood; p_aCO₂ = arterial partial pressure of carbon dioxide; p_aO₂ = arterial partial pressure of oxygen; WT = wild-type; R192Q = homozygous R192Q knockin mice; Aged = 11-13 months old.

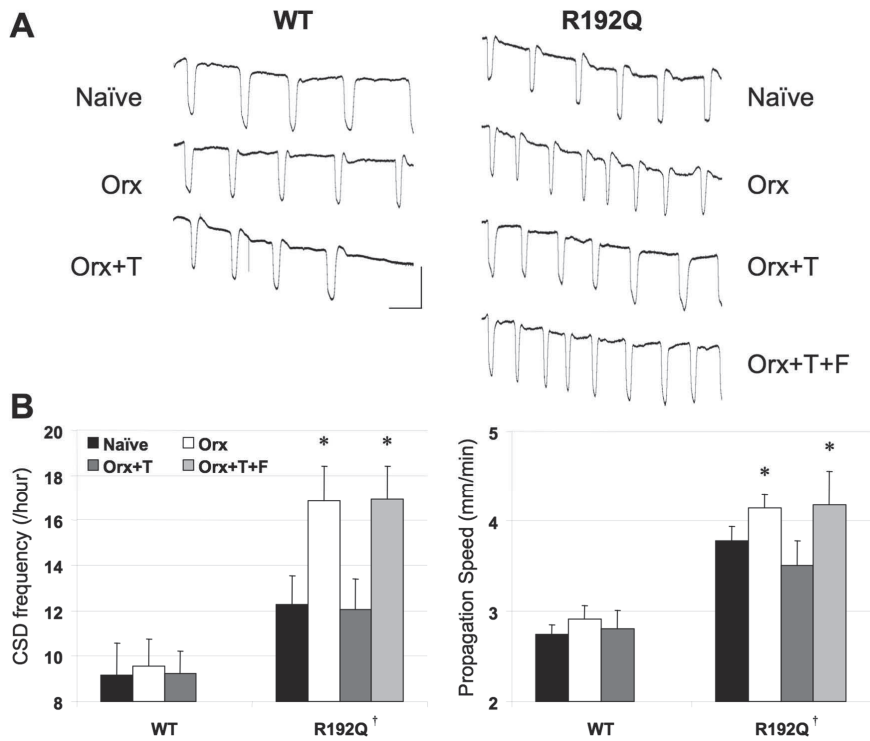


Figure. Androgenic modulation of CSD in R192Q mutant mice. (A) Representative electrophysiological recordings from male wild-type (WT) and homozygous R192Q mutant mice showing repetitive CSDs evoked by topical KCl application (300 mM) for 30 minutes. (B) Graphic representation of CSD frequency and propagation speed in WT and R192Q mutant mice. Naïve R192Q mutant mice developed higher frequency of CSDs compared to WT. Orchiectomy (Orx) further increased CSD frequency in the R192Q mutant, which was restored to the level of naïve R192Q mutants by chronic testosterone replacement (T). The androgen receptor blocker flutamide (F) completely abolished the effects of testosterone replacement. Vertical bar = 20 mV; horizontal bar = 4 minutes. Data are mean $\pm$ SD; * p < 0.001 vs. naïve and Orx+T R192Q mutant; † p < 0.001 vs. WT. Numbers of mice for each group are shown in the Table.

DISCUSSION

We showed that testosterone, acting via androgen receptors, suppresses genetically-enhanced CSD susceptibility. CSD suppression required chronic androgen replacement. We recently showed that estradiol augmented genetically-enhanced CSD susceptibility in FHM1 knockin mice.⁷ To the extent that mice homozygous for the FHM1 allele represent the human condition, the data suggest that estrogen and androgen exert reciprocal effects on CSD susceptibility, providing a dual mechanism that may account for the female preponderance of migraine.

Observational studies suggest that methyltestosterone and danazol, a synthetic testosterone derivative, may decrease attack frequency and severity in migraineurs.^{14-16,18,19} As androgens are known to downregulate estrogen receptor expression²⁰ and danazol also inhibits ovarian sex hormone production, it is unclear whether the clinical effects of danazol are a direct result of androgen receptor activation or secondary to suppression of estrogen actions on excitability.^{10,21,22} Complete cessation of migraine with aura attacks was reported in men treated with gonadotrophins for infertility, further implicating androgens secreted by the testes.²³ In a small cohort of male-to-female transsexuals, the prevalence of migraine with aura increased during anti-androgen combined with estrogen therapy to levels similar to that seen in females.²⁴

Unlike estrogens, the influence of androgens on neuronal structure and function has not been studied in detail. There are data suggesting that androgens modulate both presynaptic and postsynaptic mechanisms. For example, orchiectomy enhances spontaneous acetylcholine release (ie, increased frequency of miniature endplate potentials) at the neuromuscular junction possibly related to altered expression and function of VGCCs (eg, $Ca_v2.2$).²⁵ Although a specific modulation of $Ca_v2.1$ channels has not been reported, similar mechanisms may be operational at the glutamatergic central synapses. Postsynaptic glutamate receptors, particularly the *N*-methyl-D-aspartic acid (NMDA) subtype, are critical for the propagation of CSD. The nonaromatizable androgen, 5 α -dihydrotestosterone (5 α DHT), modulates NMDA responses in a complex manner in hippocampal slices from orchiectomized rats: despite larger NMDA-induced currents, irreversible depolarization and cell death at high NMDA concentrations were significantly inhibited by 5 α DHT.²⁶ The latter effect required 5 α DHT exposure times of 8 hours or more, implicating transcriptional mechanisms.

There is a well-established bidirectionally increased risk of comorbidity of migraine and epilepsy, suggesting shared underlying mechanisms.²⁷ Interestingly, there is a clinical association between androgen deficiency and epilepsy.²⁸ Consistent with this, androgens possess anticonvulsant activity in rodents by acutely enhancing γ -aminobutyric acid type A ($GABA_A$) receptor activity independent of androgen receptors.²⁹ However, we found that acute testosterone administration did not suppress CSD, and that suppression by chronic testosterone treatment was abolished by the androgen receptor blocker flutamide. Taken together with previous data suggesting that barbiturates do not significantly suppress CSD,³⁰ it is unlikely that GABAergic mechanisms play a significant role in androgenic CSD suppression.

In our study, orchiectomy and testosterone modulated CSD only in FHM1 mutant mice, and not in the WT. The mechanisms of interaction between gonadal hormones and the mutant $Ca_v2.1$ channels are not known; however, the need for chronic treatment with testosterone implicates mechanisms linked to gene expression and, possibly,

ultrastructural changes. Presynaptic, postsynaptic and astrocytic mechanisms may all be involved in the interaction between gonadal hormones and FHM1 mutations. The clear female preponderance in clinical migraine strongly suggests a reciprocal modulation of yet unidentified polygenetic migraine susceptibility factors by androgen and estrogen.

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