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Regular Article

Stress hormone corticosterone enhances susceptibility to cortical spreading depression in familial hemiplegic migraine type 1 mutant mice

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ABSTRACT

Stress is a putative migraine trigger, but the pathogenic mechanisms involved are unknown. Stress and stress hormones increase neuronal excitability by enhancing glutamatergic neurotransmission, but inhibitory effects have also been reported. We hypothesise that an acute rise in stress hormones, such as corticosteroids which are released after stress, increase neuronal excitability and thereby may increase susceptibility to cortical spreading depression (CSD), the mechanism underlying the migraine aura. Here we investigated effects of acute restraint stress and of the stress hormone corticosterone on CSD susceptibility as surrogate migraine marker, in a transgenic mouse model of familial hemiplegic migraine type 1 (FHM1), which displays increased glutamatergic cortical neurotransmission and increased propensity for CSD. We found that 20-min and 3-h restraint stress did not influence CSD susceptibility in mutant or wild-type mice, despite elevated levels of plasma corticosterone. By contrast, subcutaneous administration of 20 mg/kg corticosterone increased CSD frequency exclusively in mutant mice, while corticosterone plasma levels were similarly elevated in mutants and wild types. The effect of corticosterone on CSD frequency was normalised by pre-administration of the glucocorticoid receptor (GR) antagonist mifepristone. These findings suggest that corticosteroid-induced GR activation can enhance susceptibility to CSD in genetically susceptible individuals, and may predispose to attacks of migraine. Although corticosterone levels rise also during acute stress, the latter likely triggers a spatiotemporally more complex biological response with multiple positive and negative modulators which may not be adequately modeled by exogenous administration of corticosterone alone.

Introduction

Migraine is a common disabling brain disorder typically characterised by recurring attacks of severe head pain and associated symptoms of autonomic and neurological dysfunction (Goadsby et al., 2002; IHCD, 2004). In one-third of patients, attacks are associated with neurological aura symptoms (Launer et al., 1999). Migraine auras are likely caused by cortical spreading depression (CSD) that is defined as a slowly spreading cortical wave of neuronal and glial depolarisation (Lauritzen, 1994). Hyperexcitability of the cortex has been described in migraine patients (Aurora and Wilkinson, 2007) compared to healthy controls, but much less is known how migraine attacks come about. It is still unclear whether acute stress is in fact one of the trigger factors for attacks, although often reported by patients (Hauge et al., 2011; Sauro and Becker, 2009). Moreover, it is unknown *how* hormones that are released upon stress may precipitate attacks (Borsook et al., 2012).

Corticosteroid hormones (cortisol in humans and corticosterone in rodents), which are released in high amounts after stress, act by binding to mineralocorticoid (MR) and glucocorticoid receptors (GR) and are known to increase neuronal excitability (Joels et al., 2012; Popoli et al., 2011). Unlike MRs, GRs are quite abundantly expressed in several layers of the cerebral cortex, and GR pathways are known to mediate excitatory effects of stress hormones on neurotransmission, particularly after acute stress (Joels and Baram, 2009). It is therefore plausible that possible effects of stress hormones on cortical excitability in the migraine brain are GR mediated.

Abbreviations: CSD, cortical spreading depression; FHM type 1, familial hemiplegic migraine type 1; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; CRH, corticotropin-releasing hormone.

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Earlier we generated transgenic knock-in mice with an R192Q missense $Ca_V2.1$ (P/Q-type) Ca^{2+} channel mutation (van den Maagdenberg et al., 2004), identified in patients with Familial Hemiplegic Migraine type 1 (FHM1; Ophoff et al., 1996); these mice are considered a relevant model of migraine. We have shown that the enhanced susceptibility to CSD in mutant animals (Eikermann-Haerter et al., 2009; van den Maagdenberg et al., 2004) was caused by increased cortical glutamatergic neurotransmission (Tottene et al., 2009). In the present study, we used CSD as a migraine-relevant readout for stress-induced changes in cortical hyperexcitability in the FHM1 R192Q mouse model. We investigated whether acute moderate or severe restraint stress may further enhance CSD susceptibility in R192Q mice and whether corticosteroid activation of GR pathways has comparable effects. Our findings provide insight into the mechanisms by which corticosteroids could contribute to triggering migraine attacks via influencing CSD susceptibility.

Materials and methods

Animals

Male homozygous *Cacna1a* FHM1 R192Q knock-in ("R192Q") and wild type ("WT") mice of 2-4 months were used. The knock-in mice were generated as previously described by introducing the human FHM1 pathogenic R192Q mutation in the orthologous mouse *Cacna1a* gene using a gene targeting approach (van den Maagdenberg et al., 2004). Mice were assigned to the different experimental groups: (i) 20-min restraint, (ii) 3-h restraint, (iii) untreated, (iv) corticosterone, (v) vehicle, (vi) mifepristone + vehicle or (vii) mifepristone + corticosterone. For each of these experimental paradigms, WT and R192Q mice were tested, separately. For most experimental groups, a sample size of 8 animals was used per genotype, except for the WT untreated ($N = 10$), WT 20-min restraint ($N = 7$) and the R192Q corticosterone + THDOC group ($N = 6$). All experiments were approved by the Animal Experiment Ethics Committee of Leiden University Medical Center.

Assessment of corticosterone plasma levels

Mice were habituated to single housing for at least 4 days after which baseline blood samples from the tail (20 μL) were collected at 10:00 a.m. four days before follow-up procedures. Corticosterone plasma levels were determined by a commercial radioactive immunoassay (MP Biomedicals Inc., Costa Mesa, CA) according to manufacturer's instructions (Sarabdjitsingh et al., 2010).

Restraint stress paradigms

Restraint stress experiments were performed in R192Q and WT mice using a single restraint paradigm (Sarabdjitsingh et al., 2012) starting between 10:00 and 10:30a.m. Mice were restraint in custom made Plexiglas cylinders (3 cm diameter) (i) for a single period of 20 min (moderate restraint) and then returned to the home cage for nearly 3 h, after which they were prepared for CSD surgery, or (ii) for 3 h (severe restraint), after which they were immediately prepared for CSD surgery. Blood samples for corticosterone plasma measurements were collected prior to the start of CSD surgery (i.e., 3 h after the end of the 20-min restraint or immediately after the 3-h restraint procedure) and at the end of CSD recordings. The immediate effect of moderate restraint stress on corticosterone plasma levels (Table 1) was determined in separate groups of R192Q and WT mice by collecting blood samples from the tail 30 min after the end of the 20-min restraint period. In pilot studies, a separate group of animals was weighed ("handled controls") after which blood samples were collected from the tail 30 min later. Since these handled controls showed slightly elevated levels of plasma corticosterone (data not shown), untreated mice were used as controls for the CSD experiments in restraint mice.

Corticosterone, mifepristone and tetrahydrodeoxycorticosterone injections

On the day of the CSD experiment, corticosterone (Sigma-Aldrich, St. Louis, MO; 20 mg/kg, in arachidonic oil) or vehicle was subcutaneously injected between 10:00 and 10:30 a.m. when endogenous corticosterone levels are low (Table 1) after which the mouse was returned to its home cage. A tail blood sample was collected 3 h after corticosterone or vehicle injection just before surgical preparation. Surgery for CSD measurements started 3 h after corticosterone or vehicle injection. Mifepristone (10 mg/kg; RU486, Sigma-Aldrich) diluted in 1,2-propanediol was injected subcutaneously 50 min prior to corticosterone/vehicle injection. Tetrahydrodeoxycorticosterone (THDOC; Sigma-Aldrich) was first diluted in 45% hydroxypropyl-β-cyclodextrin (Sigma-Aldrich) in distilled water before further dilution in 0.9% saline, and was injected intraperitoneally at 20 mg/kg shortly after the start of surgery for CSD measurements, approximately 3 h after corticosterone (20 mg/kg subcutaneously) injection.

CSD recordings under physiological control

CSD susceptibility measurements were performed as described in detail elsewhere (Eikermann-Haerter et al., 2009), under 1% isoflurane anesthesia in 20% O_2/80% N_2O with full physiological control (i.e., using a femoral artery lead for continuous blood pressure

Table 1
Corticosterone plasma levels in WT and R192Q mice at baseline, after a 20-min and 3-h restraint stress paradigm.

Time	WT	R192Q	WT 20 min restraint	R192Q 20 min restraint	WT 3 h restraint	R192Q 3 h restraint
Baseline	14.3 [6,38] *(N = 11)*	15.7 [6,96] *(N = 9)*				
30 min			110# [81,147] *(N = 4)*	165# [123,212] *(N = 5)*		
3 h			59 [14,76] *(N = 6)*	27 [6,76] *(N = 8)*	372# [251,556] *(N = 8)*	349# [232,621] *(N = 8)*
Post-CSD			231 [164,271] *(N = 5)*	260 [232,338] *(N = 8)*	253 [155,401] *(N = 8)*	195 [165,694] *(N = 8)*

Values are corticosterone plasma levels in ng/mL, shown as medians with [minimum, maximum] values; group sizes are indicated in italics. Corticosterone plasma levels were determined from tail blood. Blood samples were collected from R192Q and WT mice at baseline, 30 min and 3 h after 20-min restraint stress and immediately after 3-h restraint stress. Pairwise comparisons were made using Mann–Whitney *U*-test corrected for multiple testing (adjusted *p*-value 0.008). Significant differences compared to baseline are indicated with # (Mann–Whitney). Note that 3 h after 20-min restraint stress corticosterone plasma values had decreased to baseline levels, as indicated by the lack of a significant difference between baseline and the 30 min after 20-min restraint samples. There were no differences between R192Q and WT corticosterone plasma levels at any of the time-points.

monitoring and blood sampling, and tracheotomy for artificial ventilation). Arterial blood gases (pCO_2, pO_2) and pH were measured at the start and end of CSD recordings and were maintained within normal limits by adjusting ventilation when necessary (Table 3). For CSD susceptibility measurements, the mouse was transferred into a stereotactic frame after which the skull was exposed and 2 burr holes were prepared over the right hemisphere for (i) CSD recording from the motor cortex (0.5 mm anterior from bregma; 2 mm lateral) and (ii) CSD induction on the occipital cortex (3.5 mm posterior, 2 mm lateral). CSD was induced by application of a cotton ball soaked in 300 mM KCl on the occipital cortex for 30 min while DC-potential recordings were made from the motor cortex (Fig. 1). Data were sampled (200 Hz), amplified (10×) and low-pass filtered at 4 Hz and analysed offline using LabChart (AD Instruments, Colorado Springs, CO).

Statistical analysis

Data are presented as median (corticosterone plasma data and CSD latency) or mean ± SD. Statistics were calculated using SPSS (version 17; SPSS Inc., Chicago, IL). Effects of moderate (20 min) and severe (3 h) restraint stress on CSD frequency (Fig. 1) as well as the effect of genotype and corticosterone treatment on CSD frequency (Figs. 2A, B) were tested with 2-way ANOVA and post hoc Bonferroni correction. The effect of corticosterone and THDOC in R192Q mice was tested with 1-way ANOVA and post hoc Bonferroni in comparison to the R192Q corticosterone and R192Q vehicle groups only. Systemic physiological data and CSD amplitude and duration (Table 3) were compared among groups using 1-way ANOVA and Bonferroni correction. For skewed parameters (corticosterone plasma data and CSD latency), corresponding non-parametric tests were used (Kruskal–Wallis, followed by Mann–Whitney *U*-test). Significance was set at 0.05 and corrected for multiple testing where applicable.

Results

Acute restraint stress does not influence CSD frequency in FHM1 R192Q and WT mice

We investigated whether an acute moderate or severe stressor influences CSD susceptibility in FHM1 mice, by using a single-restraint stress paradigm of 20 min or 3 h duration in separate groups of R192Q and WT mice, with untreated mice as controls. Plasma corticosterone levels that were determined 30 min after the end of a moderate stressor (20-min restraint) were elevated compared to baseline in both R192Q and WT mice, with no difference between genotypes. Shortly before the start of the CSD experiments, i.e., 3 h after the end of the 20-min restraint period, corticosterone plasma levels had normalised to baseline values in both R192Q and WT mice (Table 1). We observed no effect of the

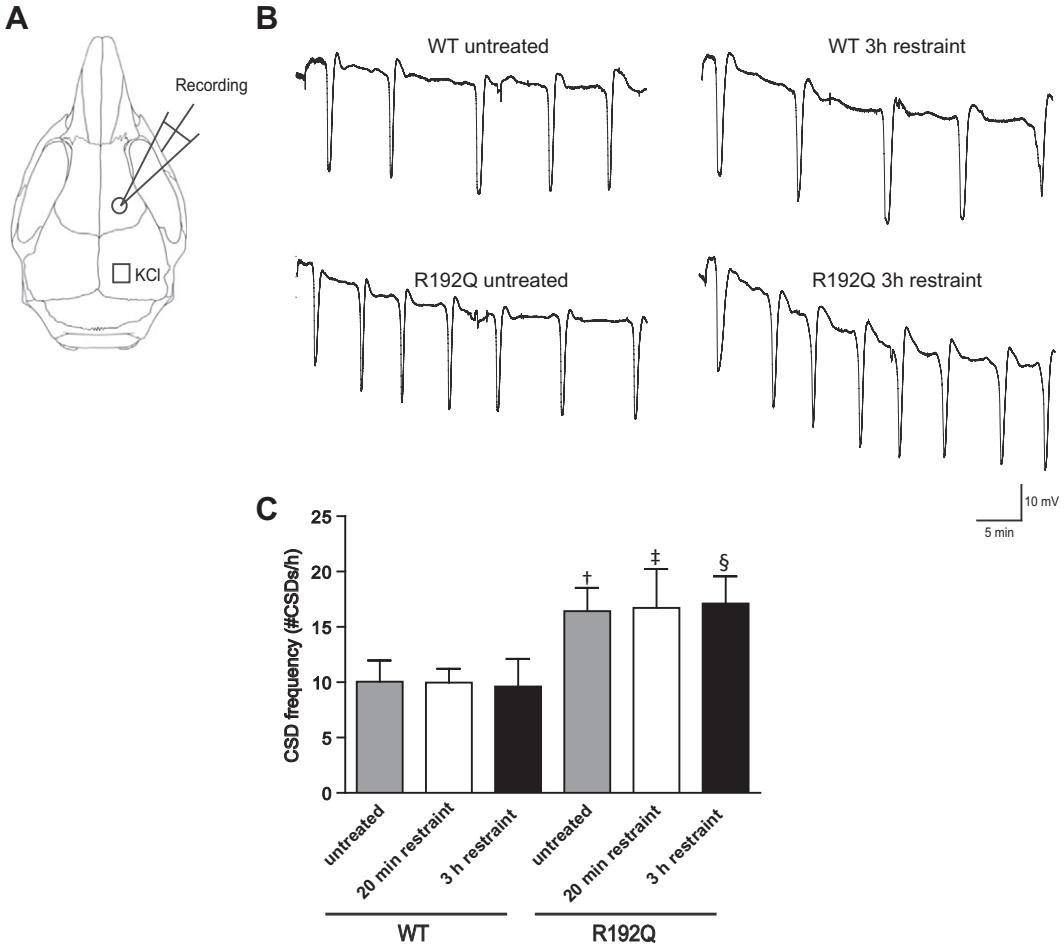

Fig. 1. Moderate or severe restraint stress does not influence CSD susceptibility in FHM1 R192Q mice. (A) Schematic of the CSD induction site (KCl application) on the occipital cortex and DC-recording site in the motor cortex. (B) Specimen recordings showing CSD events measured in untreated and 3 h restraint groups of WT and R192Q mice, illustrating comparable CSD frequencies in untreated compared to the 3-h restraint animals. Scale bar applies to all traces. (C) Bar diagram (mean ± SD) showing CSD frequency (number of CSDs/h) results from CSD recordings carried out 4 h after a 20 min (moderate) and 1 h after a 3 h (severe) restraint stress in WT and R192Q mice (WT untreated $N = 10$; R192Q untreated $N = 8$; WT 20 min restraint $N = 6$; R192Q 20 min restraint $N = 8$; WT 3 h restraint $N = 8$; R192Q 3 h restraint $N = 8$). Moderate or severe restraint stress did not influence CSD susceptibility in R192Q or WT mice (2-way ANOVA), suggesting that other neurosteroids released during stress may counteract effects of corticosterone on CSD susceptibility in R192Q mice. CSD frequency was increased in R192Q mice compared to WT mice in all treatment groups (untreated $^{\dagger}p = 0.000$; 20 min restraint $^{\ddagger}p = 0.000$; 3 h restraint $^{\S}p = 0.000$).

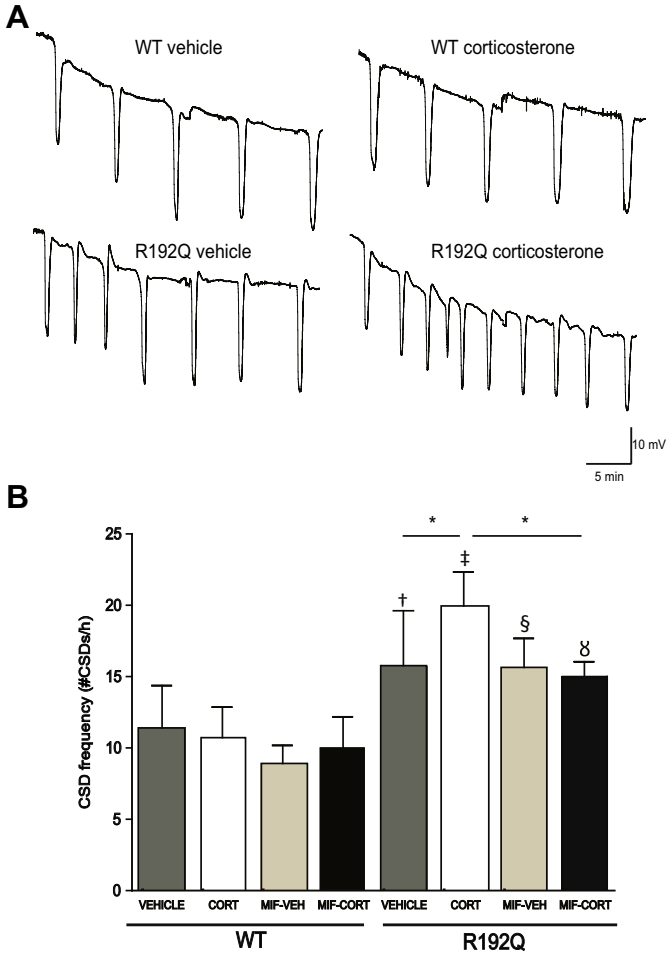

Fig. 2. Corticosterone enhances CSD susceptibility in FHM1 R192Q mutant mice via glucocorticoid receptor activation. (A) Specimen recordings showing CSD events measured in the different treatment groups of WT and R192Q mice, illustrating enhanced CSD frequency in R192Q mice that were subcutaneously injected 4 h earlier with 20 mg/kg corticosterone (CORT). Scale bar applies to all traces. (B) Bar diagram (mean ± SD) showing CSD frequency (number of CSDs/h) results from R192Q and WT mice in the different treatment groups ($N = 8$ mice/group; except for mifepristone pretreated mice injected with vehicle, MIF-VEH which are $N = 7$ mice/group). Corticosterone administration enhanced CSD frequency in R192Q mice only ($^{*}p = 0.02$ vs R192Q VEH), an effect that was prevented by pre-administration of the GR antagonist mifepristone ($^{*}p = 0.004$ vs R192Q CORT). There was a significant main effect on CSD frequency of genotype, corticosterone treatment and the interaction between genotype and treatment. Note the higher CSD frequency in the R192Q vehicle group compared to WT vehicle-injected mice ($^{\dagger}p = 0.025$), in line with earlier findings from unchallenged R192Q and WT mice (Eikermann-Haerter et al., 2009), as well as the higher CSD frequency for R192Q compared to WT mice for the CORT treated ($^{\ddagger}p = 0.000$), MIF-VEH treated ($^{\S}p = 0.000$) and MIF-CORT treated mice ($^{\S}p = 0.003$).

20-min restraint procedure on CSD frequency in R192Q mice (R192Q untreated: 16.6 ± 1.9 CSDs/h, $N = 8$ vs R192Q 20-min restraint 16.7 ± 3.5 CSDs/h, $N = 8$) nor in WT mice (WT untreated: 10.0 ± 1.9 CSDs/h, $N = 10$ vs WT 20-min restraint 10.0 ± 1.1 CSDs/h, $N = 7$; Fig. 1). CSD latency (min) was reduced in R192Q untreated compared to WT untreated mice but was not influenced by the 20-min restraint procedure (Table 3).

In contrast to 20 min restraint stress, a more severe 3 h restraint stress caused increased corticosterone plasma levels compared to baseline levels just prior to CSD measurements (Table 1). In both R192Q and WT, this severe stressor resulted in corticosterone plasma levels close to those obtained after corticosterone injection (Table 2). As with the 20-min paradigm, 3 h restraint stress did not influence CSD frequency compared to untreated controls in R192Q mice (R192Q untreated: 16.6 ± 1.9 CSDs/h, $N = 8$ vs R192Q 3-h restraint 17.1 ± 2.4 CSDs/h, $N = 8$) or WT (WT untreated: 10.0 ± 1.9 CSDs/h, $N = 10$ vs WT 3 h restraint 9.7 ± 2.5 CSDs/h, $N = 8$; Fig. 1). CSD latency (Table 3) was also not influenced by the restraint procedure compared to untreated mice.

After both the 20-min and 3-h restraint procedure, CSD duration (s) and amplitude (mV), as well as systemic physiological parameters (i.e., blood pressure, pH, pCO_2, pO_2) were similar to values from untreated animals except for a higher pCO_2 (mm Hg) value in WT untreated compared to R192Q 3-h restraint mice and a lower MABP (mm Hg) in R192Q 3-h restraint compared to WT 3-h restraint mice (Table 3).

Corticosterone administration increases CSD frequency exclusively in FHM1 R192Q mice via GR activation

We next investigated the acute effect of the stress hormone corticosterone on CSD susceptibility, by administering a dose of 20 mg/kg corticosterone in the morning 3 h before surgery was started to determine CSD frequency in R192Q and WT mice. Corticosterone plasma levels at baseline and after injection of corticosterone were comparable between R192Q and WT mice. In both genotypes, corticosterone injection resulted in strongly elevated corticosterone plasma levels, while no significant plasma corticosterone increase was observed after injection of vehicle (Table 2).

We observed a significant main effect on CSD frequency of genotype ($F[1,54] = 105,98$; $p = 0.000$), corticosterone treatment ($F[3,54] = 5,22$; $p = 0.003$), and the interaction between genotype and corticosterone treatment ($F[3, 54] = 3.39$ $p = 0.024$). Post hoc testing revealed that corticosterone injection resulted in a significantly increased CSD frequency in R192Q mice (19.9 ± 2.3 CSDs/h; $N = 8$) compared to vehicle (15.6 ± 3.8 CSDs/h; $N = 8$; $p = 0.02$), while there was no effect of corticosterone in WT mice (WT corticosterone: 10.7 ± 2.1 CSDs/h; $N = 8$; WT vehicle 11.4 ± 2.9 CSDs/h; $N = 8$; Figs. 2A, B).

To investigate a possible role of GR activation on the observed corticosterone effects in R192Q mice, the GR antagonist mifepristone (10 mg/kg) was injected 50 min prior to corticosterone injection.

Table 2
Corticosterone plasma levels in WT and R192Q mice in the CORT, VEH and MIF treatment groups before and after CSD experiments.

Time	WT VEH	R192Q VEH	WT CORT	R192Q CORT	WT MIF-CORT	R192Q MIF-CORT	WT MIF-VEH	R192Q MIF-VEH
3 h	33.0 [13,210]	54.4 [9,90]	466.7# [233,1055]	522.8# [407,668]	1306.2# [699,1600]	1017.3# [755,1648]	332.4 [300,354]	326.3 [58,348]
	(N = 7)	*(N = 6)*	*(N = 5)*	*(N = 4)*	*(N = 7)*	*(N = 8)*	*(N = 7)*	*(N = 7)*
post-CSD	158.9 [140,175]	195.9* [163,339]	215.4# [183,339]	301.1# [211,562]	609.6 [227,1057]	521.0 [240,739]	226.4 [199,288]	286.8 [282,354]
	(N = 8)	(N = 8)	(N = 8)	(N = 8)	(N = 8)	(N = 8)	(N = 7)	(N = 7)

Values are corticosterone plasma levels in ng/mL, shown as medians with [minimum, maximum] values; group sizes are indicated in italics. Corticosterone plasma levels were determined from tail blood. Blood samples were collected from R192Q and WT mice at 3 h after a single subcutaneous administration of 20 mg/kg corticosterone (CORT) or vehicle (VEH), with or without pretreatment with the GR antagonist mifepristone (MIF). Post-CSD samples were taken at the end of CSD recordings under isoflurane anesthesia. Group comparisons were done with Kruskal–Wallis test: $\chi^2 = 51.942$, $p = 0.000$. Pairwise comparisons were made using a Mann–Whitney *U*-test, corrected for multiple testing ($p = 0.005$). Significant increases compared to baseline (see Table 1) are indicated by # (Mann–Whitney). For corticosterone-injected animals, corticosterone plasma levels were comparable between R192Q and WT mice in the different treatment groups, including at the end of the experiment, except for post-CSD levels of vehicle-injected mice which showed slightly higher corticosterone plasma levels for R192Q compared to WT mice ($^{*}p = 0.0013$ Mann–Whitney *U*-test). Both for corticosterone and vehicle-injected animals, there was no correlation between corticosterone plasma levels at the end of surgery and CSD frequency (Spearman's $\rho = 0.192$, $p = 0.224$).

Table 3
Systemic physiological parameters and CSD characteristics in WT and R192Q mice in the different treatment groups.

Groups	*N*	pH	pCO_2 (mm Hg)	pO_2 (mmHg)	MABP (mmHg)	CSD amplitude (mV)	CSD duration (s)	CSD latency (min)
WT untreated	10	7.38 ± 0.03	32.4 ± 1.73†	120.5 ± 12.3	83.9 ± 13.1	16.9 ± 4.1	30.4 ± 9.2	1.3 [1.2–1.5]
R192Q untreated	8	7.39 ± 0.02	31.2 ± 1.6	128.9 ± 11.5	89.8 ± 6.6	20 ± 2.8	21.7 ± 6.2	0.8§ [0.5–1.2]
WT 20 min restraint	6	7.39 ± 0.02	32.1 ± 1.9	136.3 ± 19.6	92.6 ± 6.2	18.0 ± 3.3	21.1 ± 4.5	1.3 [1.2–1.4]
R192Q 20 min restraint	8	7.38 ± 0.03	31.7 ± 1.2	129.6 ± 15.1	90.7 ± 3.8	19.6 ± 3.9	22.6 ± 3.82	1.0 [0.2–1.2]
WT 3 h restraint	8	7.37 ± 0.01	30.0 ± 2.0	123.7 ± 13.5	80.1 ± 3.5‡	14.9 ± 2.9	26.4 ± 5.8	1.2 [1.1–2.0]
R192Q 3 h restraint	8	7.36 ± 0.01	29.8 ± 1.5	128.5 ± 17.6	93.7 ± 4.5	17.1 ± 1.7	31.1 ± 6.9	1.0 [0.5–2.5]
WT VEH	8	7.37 ± 0.01	33.9 ± 2.5	127.9 ± 15.1	80.5 ± 6.0	21.7 ± 3.3	23.9 ± 6.0	1.4 [1.3–2.4]
R192Q VEH	8	7.36 ± 0.03	32.2 ± 4.0	119.7 ± 14.3	82.2 ± 7.8	22.6 ± 1.7	22.3 ± 4.0	1.1$^{\#}$ [1.0–1.4]
WT CORT	8	7.35 ± 0.01	35.5 ± 2.0	127.9 ± 13.1	85.7 ± 6.8	21.2 ± 4.8	22.9 ± 8.6	1.5 [1.2–2.1]
R192Q CORT	8	7.33 ± 0.01*	31.9 ± 4.1	128.2 ± 19.5	82.1 ± 5.5	21.1 ± 1.9	21.6 ± 2.2	1.2$^{\#}$ [1.0–1.3]
WT MIF-VEH	7	7.35 ± 0.02	33.8 ± 3.1	127.7 ± 9	76.1 ± 5.4	18.7 ± 4.3	29.3 ± 5.8	1.4 [1.3–4.5]
R192Q MIF-VEH	7	7.37 ± 0.02	32.7 ± 3	135.6 ± 23.3	84.8 ± 7.2	20.1 ± 2.2	26.4 ± 6.3	1.0$^{\#}$ [0.5–1.0]
WT MIF-CORT	8	7.36 ± 0.02	32.3 ± 3.1	132.8 ± 09.2	79.0 ± 8.0	23.6 ± 2.8	24.5 ± 5.0	1.4 [1.3–2.1]
R192Q MIF-CORT	8	7.36 ± 0.03	32.3 ± 3.2	127.2 ± 21.4	80.2 ± 7.0	21.0 ± 3.0	25.9 ± 7.3	1.2 [1.1–2.3]

Values shown are mean ± SD. Because CSD latency values (min) were not normally distributed and variances were unequal, these are shown as median with [minimum, maximum] values. Physiological parameters during CSD frequency recordings were kept within physiological ranges. In the restraint stress-treated mice, there were no significant differences in physiological parameters except for a slightly higher pCO_2 value (mm Hg) for WT untreated (indicated by †) compared to R192Q 3-h restraint ($p = 0.028$) and a lower MABP (mean arterial blood pressure; mm Hg) in WT 3-h restraint (indicated by ‡) compared to R192Q 3-h restraint ($p = 0.013$). R192Q untreated mice had a reduced CSD latency compared to WT untreated mice ($p = 0.009^{\S}$). In the corticosterone injection experiments, there was a slightly lower blood pH in the R192Q CORT group compared to WT VEH ($^{*}p = 0.03$) and R192Q MIF-VEH ($p = 0.01$). Note that lower pH, if effective in the brain, would reduce rather than enhance neuronal excitability. Except for MIF-CORT groups ($p = 0.08$), R192Q mice showed a reduced CSD latency compared to WT across all treatment groups (significance indicated by $^{\#}$; CORT $p = 0.003$, VEH $p = 0.005$ and MIF-VEH $p = 0.0006$).

Mifepristone pre-treatment normalised CSD frequency in R192Q mice (15.0 ± 1.0 CSDs/h; $N = 8$) to the level of vehicle-controls (15.6 ± 3.8 CSDs/h; $N = 8$; Fig. 2B). Mifepristone did not influence CSD frequency in vehicle-injected R192Q mice (15.6 ± 2 CSDs/h; $N = 7$), vehicle-injected WT mice (8.9 ± 1.2 CSDs/h; $N = 7$), or corticosterone-injected WT mice (9.9 ± 2.1 CSDs/h; $N = 8$). Since the CSD frequencies of mice in the combined mifepristone with vehicle group were comparable to those in the vehicle-control group, no separate controls for mifepristone injection were included. CSD duration (s), amplitude (mV), latency (min) and physiological parameters were not influenced by corticosterone, vehicle or mifepristone injection (Table 3).

To investigate whether neurosteroids as a stress mediator may counteract effects of corticosterone on CSD frequency, we administered tetrahydrodeoxycorticosterone (THDOC), a neurosteroid with anticonvulsant properties (e.g., Kokate et al., 1994, 1996; Reddy and Rogawski, 2002) that is synthesised from corticosterone precursor 11-deoxycorticosterone (Kaminski and Rogawski, 2011), at a dosage of 20 mg/kg, 40 min prior to CSD measurements in an additional group of R192Q mice ($N = 6$) that received a 20 mg/kg corticosterone injection 3 h earlier. In these mice, corticosterone plasma levels 3 h after corticosterone injection were strongly elevated (781.3 ± 312.0 ng/mL). The additional administration of tetrahydrodeoxycorticosterone did not change CSD frequency (18.8 ± 3.1 CSDs/h; $N = 6$) compared to that observed for the group of R192Q mice injected with corticosterone alone (see above and Fig. 2, 19.9 ± 2.3 CSDs/h; $N = 8$; $p = 1$).

Discussion

Transgenic FHM1 R192Q knock-in mice display an increased susceptibility to experimentally induced CSD (Eikermann-Haerter et al., 2009; van den Maagdenberg et al., 2004) that can be explained by an enhanced cortical glutamate release (Tottene et al., 2009). The enhanced CSD susceptibility serves as a measure of excitability and surrogate migraine marker. Stress and stress hormones can also cause direct changes in glutamatergic neurotransmission, leading to increased neuronal excitability (Popoli et al., 2011), but it is not known if this can explain why acute stress may enhance propensity to migraine attacks. Our data show that acute restraint stress does not influence CSD frequency in R192Q or WT mice, despite elevated plasma corticosterone levels. Administration of the stress hormone corticosterone, however, increases CSD frequency within 3–4 h in R192Q mice and not in WT mice, without affecting blood pressure or blood-gas parameters. The corticosterone-induced increased CSD susceptibility in R192Q mice was prevented by pre-administration of the GR antagonist mifepristone. These findings illustrate that corticosterone-induced GR pathway activation can enhance susceptibility to CSD in genetically susceptible individuals and may predispose to attacks of migraine. Although corticosterone levels rise during acute stress, the latter likely triggers a spatiotemporally more complex biological response with multiple positive and negative modulators (Joels and Baram, 2009), which may not be adequately modeled by exogenous administration of corticosterone alone.

The absence of a CSD effect after a 20 min moderate restraint may be related to the fact that corticosterone plasma levels were only transiently increased with this paradigm and had returned to baseline values shortly before start of the CSD experiment. The 3 h restraint paradigm however did not influence CSD susceptibility either, in both R192Q and WT mice, despite corticosterone plasma levels that were elevated to a similar extent as observed after 20 mg/kg corticosterone injection. It is possible that the biokinetics of corticosterone in case of restraint stress do not mimic those achieved by external corticosterone administration since plasma levels do not necessarily reflect effects at the cortical or cellular level. In addition, in case of an acute stress paradigm apart from corticosterone, other stress mediators, such as neurosteroids like tetrahydrodeoxycorticosterone and allopregnanolone (Purdy et al., 1991; Zimmerberg and Brown, 1998) and the neuropeptide corticotropin-releasing hormone (CRH; Vale et al., 1981), are known to be elevated which could have interfered with the effect of corticosterone on CSD. Although tetrahydrodeoxycorticosterone and allopregnanolone have been shown to modulate the physiological response to stress by promoting GABAergic inhibitory neurotransmission (Bitran et al.,

1995; Stromberg et al., 2005) and can suppress hyperexcitability in mouse seizure models (e.g., Kokate et al., 1994; Kokate et al., 1996; Reddy and Rogawski, 2002), the administration of tetrahydrodeoxycorticosterone to R192Q mice that were earlier injected with corticosterone did not seem to affect the corticosterone-induced increase in CSD frequency in our study. This outcome is not entirely surprising, since GABAergic agonists in general do not to suppress CSD susceptibility (van Harreveld and Stamm, 1953; Brand et al., 1998; Kitahara et al., 2001). Possibly, suppressed release of the endogenous stress hormone CRH in the aftermath of stress may contribute to the lack of change in CSD frequency upon restraint stress; this would fit with the notion that high levels of CRH generally exerts excitatory actions (Blank et al., 2003).

The exact mechanisms by which corticosterone influences CSD susceptibility in FHM1 mice are unknown, but the involvement of GR suggests interaction at the level of excitatory glutamatergic neurotransmission. GR activation by stress or corticosterone has been shown to influence glutamatergic neurotransmission mainly by increasing post-synaptic glutamate responses (Karst and Joels, 2005; Yuen et al., 2009) and L-type Ca^{2+} currents (Chameau et al., 2007). The FHM1 R192Q gain-of-function mutation leads to increased Ca^{2+} influx pre-synaptically, resulting in increased glutamate release (Tottene et al., 2009; van den Maagdenberg et al., 2004). When corticosterone is administered to FHM1 R192Q mice, an additive effect of GR activation and the FHM1 $Ca_V2.1$ gain-of-function on glutamatergic transmission might cause even higher CSD frequencies than achieved by either condition alone. In WT mice, that lack the genetically increased level of glutamatergic neurotransmission, corticosterone apparently does not have sufficient effect by itself to influence CSD susceptibility.

The finding that mifepristone pre-treatment prevented the corticosterone-induced increase in CSD frequency in R192Q mice indicates that the corticosterone effect on CSD susceptibility is specific and mediated by activation of GR pathways. The corticosterone effect emerged in the time-period related to delayed effects mainly involving GR, and not MR, actions (de Kloet et al., 2005; Joels and Baram, 2009; Joels et al., 2012). Observations that mifepristone is generally ineffective in blocking membrane-receptor-mediated events (Di et al., 2003; Liu et al., 2007; Zhang et al., 2012), make it plausible that the observed GR mediated effects on CSD in R192Q mice were genomically mediated, although non-genomic actions cannot be ruled out.

In conclusion, this study showed that an acute stressor does not influence CSD susceptibility in our FHM1 mouse migraine model, while acute administration of corticosterone specifically enhances CSD susceptibility in FHM1 mutants and not in wild types. This could reflect the fact that both the FHM1 mutation and corticosterone, via GR activation, exert their effect at the level of glutamatergic neurotransmission, thus providing a possible mechanistic underpinning of their interaction. It can be hypothesised that susceptible individuals may be protected against migraine in cases of acute stress as long as other stress-induced factors counteract GR-mediated actions of corticosteroids on glutamatergic transmission. This protection may fall short when a disbalance between corticosteroids and such other stress hormones occurs. Future work should reveal the complex interplay of corticosterone with other stress mediators in the context of acute stress and CSD susceptibility, as well as mechanisms underlying effects of chronic stress (Borsook et al., 2012), as opposed to acute stress, on migraine characteristics.

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