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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.