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Original article

Potential risk of dihydroergotamine causing medication-overuse headache: preclinical evidence

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Background: Overuse of abortive medication is a common factor contributing to an increase in headache frequency in patients with migraine. Whether or not chronic exposure to dihydroergotamine (DHE) can lead to this transformation remains uncertain.
Objective: To determine the effect of acute and chronic DHE exposure on development of cortical spreading depression (CSD) and trigeminal nociception.
Methods: The study comprised two experiments, namely acute and chronic exposure. In the acute experiment, a single dose of DHE (100 μg/kg) was given to male Wistar rats after successful induction of CSD. In the chronic experiment, DHE was given daily for the period of 0, 7, 14, and 28 days. CSD was induced 30 minutes after the final injection and the cortical field potential was recorded. Expression of c-Fos in caudal brainstem was used as an indicator of trigeminal nociception.
Results: Acute exposure to DHE attenuated the expression of c-Fos in the caudal brainstem without change in CSD response. By contrast, chronic exposure (14 and 28 days) to DHE increased the area under the curve of CSD waveforms. In parallel with the change in the CSD, there was significant increase of c-Fos expression within 14 days exposure to DHE and the expression remained significantly elevated for up to the 28 days examined.
Conclusion: Our study demonstrated that chronic DHE administration can increase cortical excitability and increase c-Fos expression in caudal brainstem. Our preclinical evidence suggests the possible adverse effect of chronic DHE use in causing chronification of headache.

Keywords: Cortical excitability, cortical spreading depression, CSD, c-Fos expression, dihydroergotamine, DHE, medication-overuse headache, MOH, trigeminal nociception

Transformation of headache from an episodic to a chronic form in patients with migraine is a well-recognized phenomenon. A condition called 'medication-overuse headache' (MOH) has been included in the International Classification of Headache Disorders [1]. Overuse of abortive medication is a major factor contributing to this transformation. Epidemiological data has shown that almost every class of acute migraine medication, including ergots, triptans, and analgesics, is able to cause MOH if used excessively [2, 3].

Dihydroergotamine (DHE) was introduced as an abortive migraine medication in 1945 [4]. Like the ergot alkaloids, DHE binds to various receptors, including those for serotonin (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2C}), noradrenaline (α_{1a}, α_{1b}, α_{2a}, α_{2b}, α_{2c}, β_3), and dopamine (D_2, D_3, D_4) [5].

DHE has poor oral availability (less than 1%) [6]. Therefore, to improve its efficacy, this drug should be given parenterally. Although intravenous, intramuscular, and subcutaneous DHE are effective in aborting migraine attack, these parenteral routes are considered invasive and not suitable for patients to use for self-medication. As a result, the probability of overusing DHE by these methods of administration is minimal. However, an orally inhaled DHE has recently been introduced. A clinical trial assessing the efficacy of an orally inhaled DHE showed its clinical efficacy over placebo [7]. Its efficacy and convenience allows the patients to self-medicate. This increases the possibility of overuse of this compound, especially in those who suffer from frequent attacks. Whether or not chronic DHE exposure can lead to MOH remains uncertain.

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The objective of this study was to investigate whether chronic exposure to DHE can lead to MOH as assessed in a rat model of headache. We designed experiments according to our previous studies of chronic analgesic exposure. In those studies, we showed that animals with prolonged analgesic exposure demonstrated an increased susceptibility to develop cortical spreading depression (CSD), an electrophysiological phenomenon analogous to the aura phase of migraine [8, 9]. CSD activates the trigeminovascular system, including the large cranial vessels, proximal intracranial vessels, and the dura mater that are innervated by branches of the ophthalmic division of the trigeminal nerve [10]. Peripheral trigeminal activation leads to the stimulation of neurons in the superficial layers of the trigeminal nucleus caudalis (TNC), and then results in a primary headache. In this study we compared the effects of acute and chronic administration of ergot alkaloids on the development of CSD in rat brains. We also compared the trigeminal nociceptive response using expression of c-Fos in lower brainstem as an indicator.

Materials and methods

Animal preparation

Adult male Wistar rats weighing 250–350 g were used in the experiments and were purchased from the National Laboratory Animal Center, Mahidol University, Nakorn-Pathom, Thailand. Rats were housed in stainless steel cages in a ventilated room under a 12-hour dark–light cycle, and were allowed access to food and water ad libitum. All of the protocols used in this study were approved by the Animal Care and Use Committee, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Rats were anesthetized by an intraperitoneal injection of 60 mg sodium pentobarbital (Ceva Sante Animale, Libourne, France) per kg of body weight. The level of anesthesia was closely monitored throughout the experiment and tested by lack of responsiveness to a tail pinch. A tracheotomy was performed for ventilation. Cannulation of the right femoral vein was performed for intravenous infusion of 0.9% normal saline and drugs. Blood pressure and blood gas were periodically monitored by catheterization of a femoral artery using arterial catheter (RFA-01, SAI Infusion, Libertyville, IL, USA), transducer (MLT844, ADInstruments, Dunedin, New Zealand), PowerLab data acquisition system (ML765, ADInstruments) and animal ventilator (Harvard Model 683, Harvard Apparatus, MA, USA). Membranes and extremities were monitored for signs of cyanosis, and only data from healthy subjects was reported.

Drugs administration

The study was divided into two experiments. The first experiment was to investigate the effect of acute DHE administration on CSD development and trigeminal nociception. In the first experiment, rats were divided into an acute DHE group (n = 14) and an acute control group (n = 9). The acute DHE group received 100 μg/kg of DHE (Tocris Bioscience, Bristol, UK), intravenously after the third depolarization wave of CSD was presented. DHE was dissolved in 0.1% dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) at 100 mg/ml, and diluted in normal saline (100 μg/ml DHE). The dilution can attenuate protein plasma extravasation in the trigeminal ganglion. The control group was given the 0.1% DMSO as vehicle in the same volume and condition. The second experiment was to investigate the effect of chronic DHE administration on CSD development and trigeminal nociception. In the second experiment, rats were divided into four subgroups each representing different treatment times. A once daily intraperitoneal injection of DHE at 100 μg/kg was administered to different groups to represent chronic DHE treatment for 0 days (n = 11), 7 days (n = 11), 14 days (n = 12), and 28 days (n = 16) and 0.1% DMSO similarly administered to chronic control groups (n = 9, 8, 7, and 8, respectively). CSD was induced thirty minutes after the last administration of 0.1% DMSO or DHE in 0.1% DMSO.

In all experiments, CSD was induced by topical application of 3 mg solid KCl. A cortical depolarization wave of CSD presented as a result of the application. Electrocorticograms were recorded using an AcqKnowledge system version 3.7.3 (Biopac, Goleta, CA, USA) for 2 hours after acute administration and 1 hour after the final chronic administration. Following CSD induction and recording, rats were euthanized. The caudal brainstem and upper cervical cord that contain trigeminal nucleus caudalis (TNC) was isolated from the brain for western blotting.

Electrophysiology to record CSDs

Rats were placed in a stereotaxic frame; their heads fixed using ear bars. Skin and connective tissue was then removed to allow a craniotomy. Two

craniotomies (each 2 mm in diameter) were prepared using a dental drill (NSK, Tokyo, Japan). The frontal opening (1 mm anterior and 3 mm lateral from bregma) was for inserting a capillary microelectrode (Sutter Instruments, Novato, CA, USA). The parietal opening (7 mm posterior and 3 mm lateral from bregma) was prepared for CSD induction using cortical administration of solid KCl. The dura mater was carefully opened to expose the cortical surface. A capillary microelectrode filled with 4M NaCl, was inserted into the cortex at 500 μm depth using a hydraulic micromanipulator (Narishige, Tokyo, Japan). An Ag/AgCl electrode was placed on the rat skin as a reference. The variables, which included the number of peaks and area under the curve (AUC, the whole area of each waveform; mV.s) of waves within 2 hours for acute DHE administration and 1 hour for chronic DHE administration, were analyzed.

Western blotting

After recording the electrocorticograms, rats were euthanized using an excessive dose of sodium pentobarbital, and the TNC (obex to C1 level in the cervical spinal cord) was isolated. The ipsilateral TNC was homogenized in lysis buffer (10× RIPA buffer; Cell Signaling Technology, Beverly, MA, USA) with 100× protease inhibitor cocktail (Cell Signaling Technology), and centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was removed and stored at –80°C. The protein concentrations were quantified using a bicinchoninic acid-based protein assay kit (Thermo Scientific, Rockford, IL, USA). Proteins (30 μg) were denatured at 95°C for 10 minutes, and loaded onto SDS-polyacrylamide gels where they were separated by electrophoresis. After running the gels, protein was transferred to polyvinylidene difluoride membranes (GE Healthcare Life Sciences, Little Chalfont, UK). Mouse anti-c-Fos monoclonal antibody (Calbiochem, Darmstadt, Germany); dilution 1:1000, and anti-mouse horseradish peroxidase conjugated secondary antibody (Sigma-Aldrich); dilution 1:10000 were used for western blotting. c-Fos-immunoreactive protein bands were visualized on Amersham Hyperfilm for enhanced chemiluminescence (ECL) (GE Healthcare) using an ECL kit (Thermo Scientific). Densities of the c-Fos protein bands at 55 kDa on developed and fixed film were measured by scanning the films on an model TX220 scanner (Epson Singapore) and the resulting tagged image file format (TIFF)s interpreted using Scion Image Corporation software, version Beta 4.0.2 (Frederick, MD, USA). Signals from blotting were normalized to the β-actin at 43 kDa in the same blot.

Statistical analyses

All data are presented as mean ± standard deviation (SD). Statistical analyses were performed by using SPSS (version 17; IBM Corporation, Armonk, NY). An independent samples *t* test was used to determine differences between the DHE-treated and control groups. A one-way ANOVA (Bonferroni post hoc test) was used to detect the change among chronic DHE administration or control groups. $P < 0.05$ was considered to be statistically significant.

Results

Effects of DHE on the cortical activity

A series of depolarization waves of CSD was generated after the administration of solid KCl. Acute DHE administration did not affect cortical activity (**Figure 1**). Within the 2-hour recording time used for the acute DHE and control groups, the mean number of CSD waves was not significantly changed 18.36 ± 3.30 and 18.14 ± 5.76 (*t* test, $P = 0.91$, n = 14 and 9, respectively), and the sum of the AUC was 61.31 ± 11.64 and 52.54 ± 12.05 mV.s (*t* test, $P = 0.14$, n = 14 and 9, respectively).

The sum of the AUC significantly increased in the 14-day and 28-day chronic DHE administration groups (**Figure 2**). The AUC for the 14-day chronic DHE administration group was 45.55 ± 7.16 mV.s compared with 36.48 ± 6.31 mV.s for the control group (*t* test, $P = 0.03$, n = 12 and 7, respectively). The AUC for the 28-day chronic DHE administration group was 52.15 ± 11.02 mV.s compared with 39.46 ± 5.65 mV.s for the control group (*t* test, $P = 0.04$, n = 16 and 8, respectively). By contrast, there was no significant difference in total AUC between the DHE and control groups for the 0- and 7-day DHE administration. The AUC for the 0-day DHE administration group was 41.72 ± 7.13 mV.s compared with 41.78 ± 12.97 mV.s for the control group (*t* test, $P = 0.83$, n = 11 and 9, respectively), while the AUC for the 7-day DHE administration group was 42.43 ± 8.55 mV.s compared with 39.41 ± 9.18mV.s for the control group (*t* test, $p = 0.49$, n = 11 and 8, respectively). The results showed that the overall AUC among DHE administration groups was significantly changed (one-way ANOVA, $P = 0.02$, n = 11, 11, 12, and 16 in each group, $F = 3.555$), while AUC in control groups

was invariant (one-way ANOVA, $P = 0.20$, n = 9, 8, 7, and 8 in each group, $F = 1.670$). However, there was no change in the mean number of CSD waves between chronic DHE administration and control groups.

Effects of DHE on trigeminal nociception

The expression of c-Fos was used as a marker indicating the level of neuronal activation of second order neurons in the trigeminal nociceptive system. Using western blotting, the c-Fos protein was quantified as to provide a relative density with β-actin (**Figure 3A**). Acute intravenous DHE administration significantly attenuated the c-Fos protein expression. The relative density of c-Fos was 0.70 ± 0.07 in the control group and 0.61 ± 0.02 in the acute DHE group (*t* test, $P = 0.04$, n = 9 and 14, respectively, **Figure 3B**).

Intraperitoneal administration of DHE significantly attenuated the expression of c-Fos induced by CSD in the 0-day group as consistent with findings for the acute intravenous DHE administration group (**Figure 3C**). The relative density of c-Fos in the 0-day control was 0.62 ± 0.08 and 0.48 ± 0.10 for the 0-day DHE administration group (*t* test, $P = 0.04$, n = 9 and 11, respectively, **Figure 3D**). Parallel to the change in the CSD AUC, there was significant increase of c-Fos expression within 14 days exposure to DHE and the expression remained significantly elevated for up to the 28 days examined. The relative c-Fos density in the TNC was 0.61 ± 0.04 in the control group and 0.85 ± 0.13 after 14-day of DHE administration (*t* test, $P = 0.02$, n= 7 and 12, respectively, **Figure 3D**). The relative c-Fos density was 0.59 ± 0.06 for the control and 0.79 ± 0.13 after 28-day of DHE administration (*t* test, $P = 0.02$, n = 8 and 16, respectively, **Figure 3D**). However, there was no significant difference between the relative c-Fos density the control group 0.49 ± 0.11 compared with the relative density of 0.56 ± 0.10 after 7-day DHE administration (*t* test, $p = 0.47$, n = 8 and 11, respectively, **Figure 3D**). Examination of the western blot analysis also showed the significant changes of the overall c-Fos expression among DHE administration groups (one-way ANOVA, $P = 0.0002$, n = 11, 11, 12, and 16 in each group, $F = 15.41$), while there was no change of c-Fos expression in control groups (one-way ANOVA, $P = 0.64$, n = 9, 8, 7, and 8 in each group, $F = 0.57$).

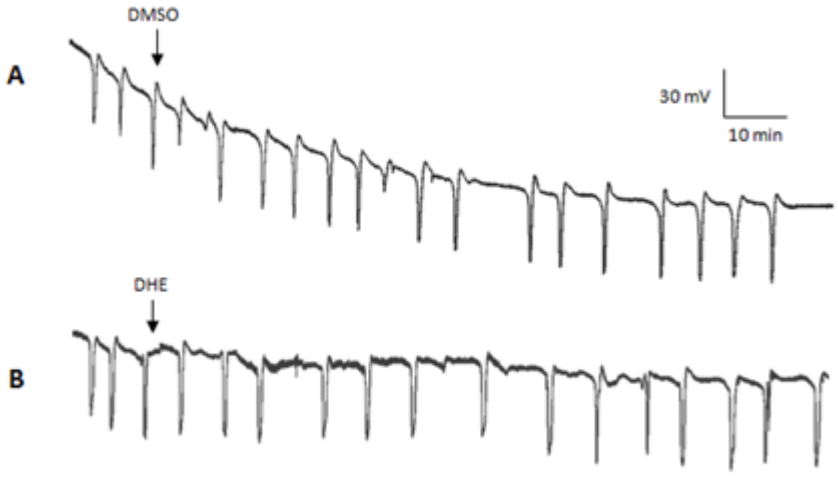

Figure 1. Effect of acute dihydroergotamine (DHE) administration on central spreading depression (CSD) development within a 2-hour period**.** The mean number and the sum of area under the curve of CSD waves were unchanged. The arrow indicates intravenous administration of dimethyl sulfoxide (DMSO, 0.1% in saline) or DHE after the third CSD cycle. **A:** acute control. **B:** acute DHE administration.

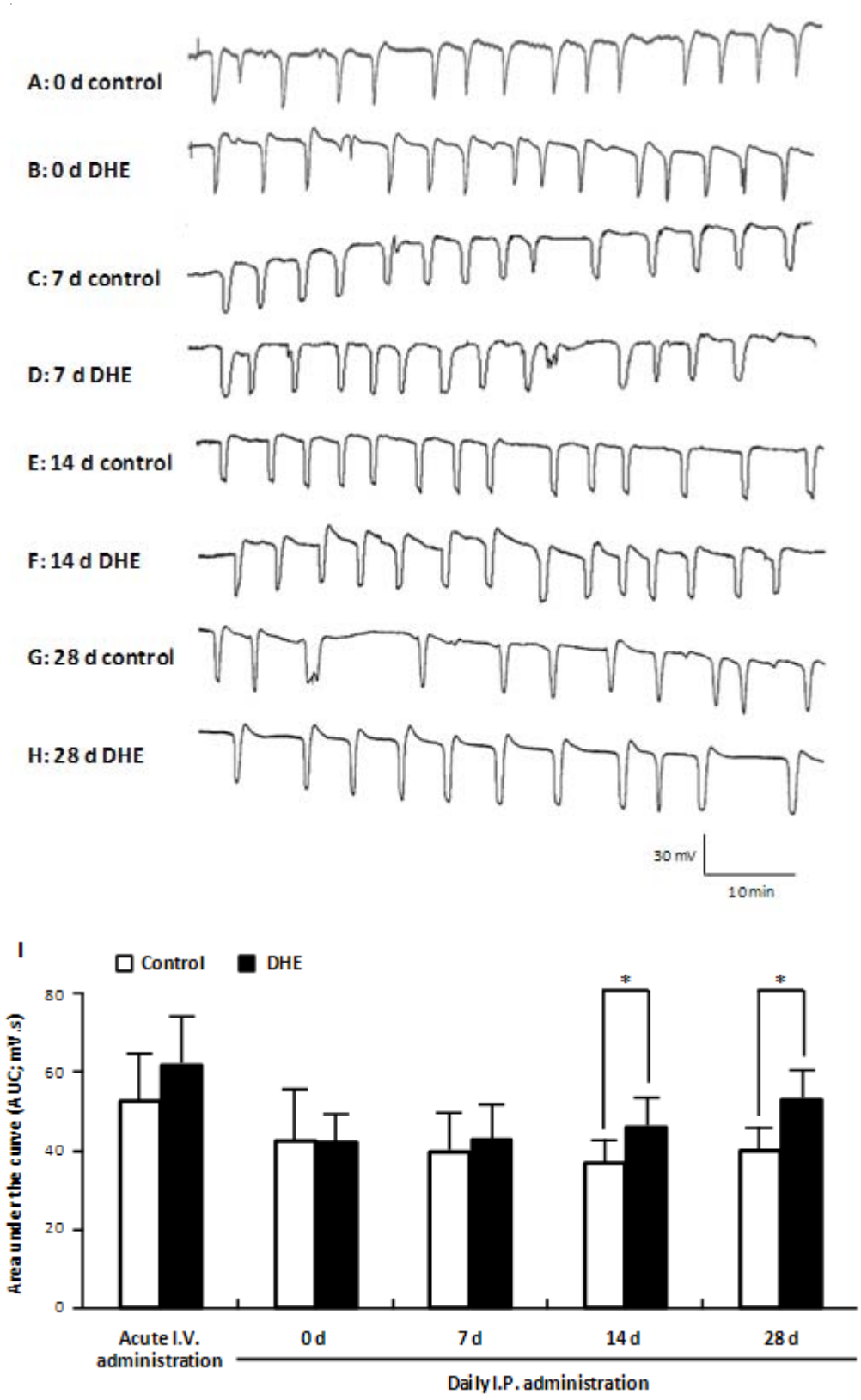

Figure 2. Effect of chronic dihydroergotamine (DHE) administration on central spreading depression (CSD) development. Chronic DHE intraperitoneal injection for 14 and 28 days leads to an increase in the AUC of CSD waves. **A:** 0-day control. **B:** 0-day DHE administration. **C:** 7-day control. **D:** 7-day DHE administration. **E:** 14-day control. **F:** 14-day DHE administration. **G:** 28-day control. **H:** 28-day DHE administration. I: Acute intravenous administration of DHE did not change the area under the curve (AUC). Whilst daily intraperitoneal administration of DHE significantly enhanced AUC of CSD within 14 and 28 days, it did not alter AUC in 0-day and 7-day of administration.

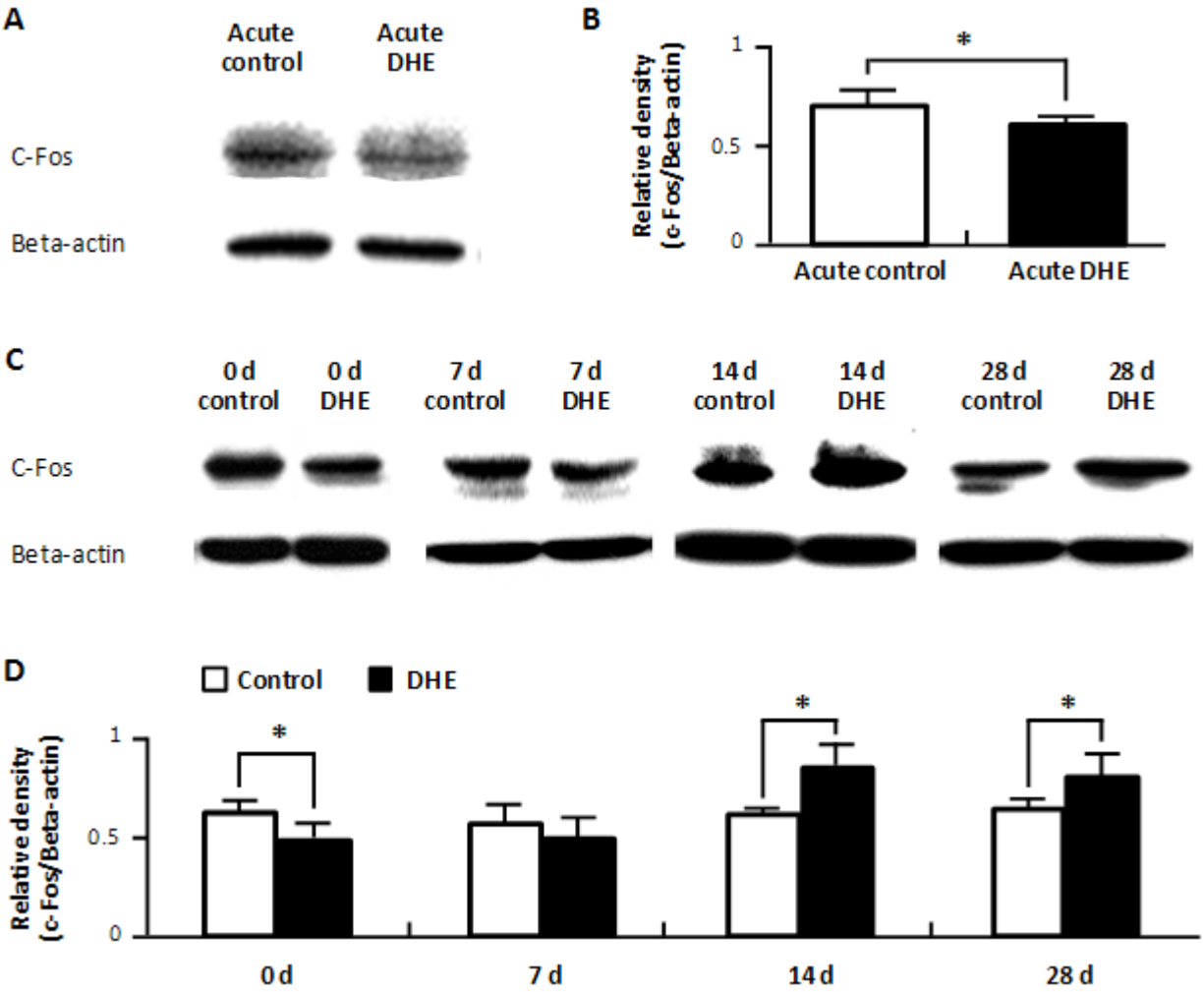

Figure 3. Molecular basis of central spreading depression-induced c-Fos protein expression in the rat trigeminal nucleus caudalis. Proteins from after treatments were analyzed using a quantitative western blotting. Chronic dihydroergotamine (DHE) intraperitoneal injection for 14 and 28 days leads to an significant increase of c-Fos expression. **A and B:** Acute intravenous DHE administration significantly attenuated c-Fos expression. **C:** Chronic intraperitoneal DHE administration altered the expression of c-Fos protein. **D:** Intraperitoneal injection of DHE attenuated c-Fos expression after 0-day, whereas it augmented c-Fos expression within 14 days of chronic administration and this level of augmentation remained significant for at least 28 days.

Discussion

The present study shows that acute and chronic exposure to DHE affects cortical excitability and the trigeminal system differently. Acute exposure to DHE attenuated the expression of c-Fos in the TNC without change in CSD response. By contrast, chronic exposure to DHE increased the AUC of CSD waveforms and CSD-evoked c-Fos expression in the caudal brainstem. These apparently paradoxical changes may reflect that chronic DHE exposure results in an increase in cortical excitability and increased trigeminal nociception.

Although we have indicated interesting findings throughout the present study, there were four issues that should be considered. First, topical application of KCl is a robust method for CSD induction where a threshold of cortical susceptibility is not assessed. Nevertheless, it is a standard protocol for inducing repetitive CSDs to evaluate the level of cortical excitability that we have used in the previous studies [8, 9, 11]. Second, we attempted to quantitate total c-Fos expression in the TNC by western blotting analysis. Western blotting offers advantages of sensitivity, specificity, and protein size determination

[12]. It is undeniable that low expression of c-Fos by weakly immunoreactive nuclei residing in the dorsal horn was also included when we have evaluated the change of c-Fos expression associated with chronic DHE treatment compared with the control groups. A further limitation is that we did not dissect out the dorsal medulla (trigeminal subnucleus caudalis) or trigeminal nucleus to enrich the potentially responsive areas and therefore apparent responses may have been dampened by c-Fos expression in irrelevant areas. Third, it is possible that chronic DHE administration alone causes an alteration of trigeminal nociception, and that the CSD intervention masks this data. Further studies are needed to examine this possibility. Fourth, the increase of AUC in CSD waves does not specifically correlate to c-Fos expression in TNC. Nevertheless, the apparently paradoxical changes may reflect that chronic DHE exposure results in an increase in cortical excitability and increased trigeminal nociception.

In our acute experiment, we found that DHE can attenuate c-Fos expression in the caudal brainstem. Our previous experiments using immunohistochemistry show that the majority of neurons in this area that express c-Fos after CSD were confined to the superficial laminae especially the TNC, an important nucleus controlling cranial nociception [8, 9]. The finding of a decrease in c-Fos expression in this area can be interpreted as a decrease in trigeminal activation and therefore nociception. This hypothesis is consistent with the antimigraine efficacy of DHE. Our experiments showed that acute DHE administration did not alter CSD generation. Administering DHE before or immediate after CSD induction showed the same results. It is known that DHE and other ergot alkaloids display affinity for adrenergic, serotonergic, and dopaminergic receptors, which probably explains its antimigraine effects [6]. Binding of DHE to adrenergic or serotonergic receptors on cranial vessels would cause vasoconstriction and might decrease the activation of perivascular nociceptors. Recent experiments showed that DHE can increase the analgesic effect of morphine and implies a central effect of ergots [13-15]. Thus, our findings suggest that abortive effect of DHE is the result of modulation of trigeminal nociception and does not rely on the modification of cortical activity.

The main findings of this study are the increase in the AUC of CSD waves and c-Fos expression in caudal brainstem after chronic DHE administration. Here, we showed that the attenuation of CSD-evoked c-Fos expression had normalized after DHE administration for 7 days. Moreover, c-Fos expression increased within 14 days of chronic DHE administration and the increase was maintained for up to 28 days during chronic administration. The increase in CSD-evoked c-Fos expression correlated well with the observed increase in the AUC of CSD. These findings simply imply the chronic DHE administration can lead to an increase in cortical excitability and facilitate trigeminal nociception. In a clinical context, chronic DHE administration can potentially cause MOH.

Although the exact mechanisms underlying MOH remain undefined, cortical hyperexcitability is a possible explanation [16, 17]. In our previous experiments, rats receiving chronic paracetamol exhibited a higher number of CSD waves, while the AUC of the waveforms was not different [8]. This pattern differed from what was observed in the present study, which showed an increased AUC of CSD waves, without a change in the number of waves. The difference between these two patterns may reflect a different pathogenesis. Increased frequency might imply an increased susceptibility to develop CSD, while an increased AUC may reflect delayed repolarization. Although alteration of the AUC relates to the change of cortical excitability reflecting the recovery cycle in migraine [18], more studies are needed to clarify the interpretation of the AUC change at a cellular level. It is noteworthy that both increased susceptibility and widening of the CSD wave have been reported in a CACNA1a knock-in migraine mouse model [19]. Some clinical studies lend support to the concept of cortical hyperexcitability. Increased sensory-evoked potentials and decreased habituation have been observed in patients with MOH [20]. The decrease in habituation can be restored in patients with MOH who are successfully treated [21]. This clinical evidence suggests that cortical hyperexcitability plays a role in the pathogenesis of this condition.

The mechanism rendering the cerebral cortex to be hyperexcitable remains to be clarified. Clinical evidence shows that both specific and nonspecific abortive migraine medication can cause MOH. Because these drugs have different pharmacological actions, it is unlikely that MOH is caused by the specific action of any single causative agent. The more likely explanation is that all drugs might share some common

mechanism in generating this phenomenon. Chronic medication may interfere with incoming nociceptive input and lead to subsequent plastic changes in both peripheral and central nociceptive systems. Deafferentation is a well-recognized mechanism generating neuropathic pain. Changes in algogenic protein expression in trigeminal ganglia and latent sensitization have been demonstrated in rats chronically exposed to triptans [22]. A derangement of the central serotonin-dependent endogenous pain control system induced by chronic medication has been proposed [16]. Observation of a decrease in serotonin in patients with MOH supports this hypothesis [23].

In conclusion, our study demonstrated that chronic DHE administration can increase cortical excitability and increase c-Fos expression in caudal brainstem. Our preclinical evidence suggests the possible adverse effect of chronic DHE use in causing MOH. Therefore, like other acute medication, the form of this compound for self-medication should be used with caution.

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