RESEARCH PAPER

Intrathecal injection of the neurosteroid, DHEAS, produces mechanical allodynia in mice: involvement of spinal sigma-1 and GABA$_A$ receptors

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Background and purpose: The neurosteroid, dehydroepiandrosterone sulphate (DHEAS) and its non-sulphated form, DHEA, are considered as crucial endogenous modulators of a number of important physiological events. Evidence suggests that DHEAS and DHEA modulate central nervous system-related functions by activating sigma-1 receptors and/or allosterically inhibiting γ-aminobutyric acic receptor type A (GABA$_A$) receptors. As both the sigma-1 receptor and the GABA$_A$ receptor play important roles in spinal pain transmission, the present study was designed to examine whether intrathecally injected DHEAS or DHEA affect nociceptive signalling at the spinal cord level.

Experimental approach: We first determined whether intrathecal (i.t.) DHEA or DHEAS injection was able to affect nociceptive thresholds to peripheral mechanical stimulation and subsequently examined whether this effect was mediated by sigma-1 or the GABA$_A$ receptors.

Key results: The i.t. DHEAS injection dose-dependently decreased the nociceptive threshold to mechanical stimulation, thus producing mechanical allodynia. Moreover, this DHEAS-induced mechanical allodynia was significantly reduced by administration of the sigma-1 receptor antagonist, BD-1047 or the GABA$_A$ receptor agonist, muscimol. Conversely, i.t. DHEA had no effect on mechanical sensitivity. However, when i.t. DHEA was combined with the GABA$_A$ receptor antagonist bicuculline, DHEA dose-dependently produced mechanical allodynia similar to that of DHEAS. This effect was blocked by BD-1047 and by muscimol.

Conclusions and implications: These findings indicate that i.t. injection of DHEAS produces mechanical allodynia and that the development of this mechanical allodynia is mediated by sigma-1 and GABA$_A$ receptors. The findings of this study raise several interesting questions for further investigations into the mechanisms underlying neurosteroid modulation of spinal pain transmission.

British Journal of Pharmacology (2009) **157,** 666–673; doi:10.1111/j.1476-5381.2009.00197.x; published online 30 April 2009

Introduction

Neurosteroids are steroid hormones that act as potent endogenous neuromodulators with rapid actions in the central and peripheral nervous systems. They are synthesized either *de novo* from cholesterol or by *in situ* metabolism of blood-borne precursors that accumulate in the nervous system independently of classical steroidogenic gland secretion rates

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(Baulieu, 1998; Compagnone and Mellon, 2000). One of these neurosteroids, dehydroepiandrosterone (DHEA) and its sulphate derivative (DHEAS) are considered as crucial endogenous modulators of numerous physiological functions, including memory, neurogenesis and aging (Baulieu and Robel, 1998; Ren *et al.*, 2004; Dillon, 2005; Maayan *et al.*, 2005; Wojtal *et al.*, 2006). Because of this, exogenous DHEA or DHEAS administration has been tried in an attempt to modulate certain neurobiological mechanisms in animals and in a few clinical trials (Baulieu, 1999; Shirayama *et al.*, 2002). For a number of neuroactive steroids, sulphation at C-3 is an important control point for the activity of endogenous neurosteroids (Park-Chung *et al.*, 1999; Gibbs *et al.*, 2006). While DHEA and DHEAS are interconversion products [DHEAS serving as the major source (60–80%) of DHEA and DHEA serving as a minor source (5–7%) of DHEAS], their actions appear to differ in several systems (Zhang *et al.*, 2002).

Recently, Kibaly *et al.* (2007) reported that acute intrathecal (i.t.) treatment with DHEA decreases the basal nociceptive thresholds in both neuropathic and control rats, suggesting that i.t. DHEA can affect spinal circuits that are involved in pain signalling. However, the role of the sulphated neurosteroid, DHEAS, on nociception in the spinal cord is unclear. It is important to note, however, that recent work indicates that neurosteroidogenesis is an endogenous mechanism activated in the spinal cord and brainstem for adaptation of the body to chronic peripheral neuropathies (Patte-Mensah and Mensah-Nyagan, 2008).

The possibility that neurosteroids, such as DHEAS and DHEA, could be endogenous activators/inactivators of the sigma-1 receptor and possibly even the 'endogenous ligands' for this receptor has generated significant interest in this area (see reviews by Dubrovsky, 2005; Maurice *et al.*, 2006). Recently, Cheng *et al.* (2008) have shown that DHEAS inhibits persistent sodium currents via the activation of sigma-1 receptors-Gi protein-protein kinase C-coupled signalling pathway, providing the first mechanism by which DHEAS may affect the excitability of neurons via the sigma-1 receptor. With respect to pain, intraplantar injection of DHEAS has been shown to induce nociception via sigma-1 receptors in the peripheral nociceptive flexor test (Ueda *et al.*, 2001). Moreover, accumulating data from our laboratories have shown that spinal sigma-1 receptors are involved with pain facilitatory mechanisms (Kim *et al.*, 20062008). Therefore, it seems likely that DHEA or DHEAS induce pain facilitation in part via activation of spinal sigma-1 receptors. On the other hand, it is well established that DHEAS and DHEA act as allosteric negative modulators of γ-aminobutyric acic receptor type A (GABA$_A$) receptors (Belelli and Lambert, 2005) and that GABAergic inhibitory activity tonically modulates spinal pain transmission (Jasmin *et al.*, 2004). Interestingly, non-sulphated DHEA is less potent than DHEAS in inhibiting GABA$_A$ receptors (Imamura and Prasad, 1998; Mehta and Ticku, 2001). Based on this information we hypothesize that there are differences between the effect of DHEAS and DHEA on spinal pain transmission. Moreover, we hypothesized that spinally administered DHEAS would induce a greater facilitatory effect on pain sensation than DHEA and that this effect occurs via activation of sigma-1 receptors and inhibition of GABA$_A$ receptors.

To verify this hypothesis, pharmacological approaches were used to study the effects, as well as the mechanism of action, of intrathecally administered DHEAS versus DHEA on mechanical nociceptive thresholds in mice. It is important to point out that the passage of DHEAS into the brain is proportionately much less than DHEA (Baulieu and Robel, 1998), but nonetheless several lines of evidence suggest that peripheral DHEAS can pass through the blood–brain barrier and affect central brain sites (Akwa *et al.*, 1993; Asaba *et al.*, 2000; Chen *et al.*, 2006). On the other hand, it has been reported that intraplantar treatment of DHEAS causes nociceptive flexor responses indicating that DHEAS also has a peripheral site of action (Ueda *et al.*, 2001). Based on these data, it is difficult to discriminate peripheral versus central (spinal/brain) actions of DHEAS or DHEA, as both sites are capable of influencing nociception. More importantly, from a mechanistic point of view, it is currently unclear how spinal DHEAS directly affects pain sensation. Therefore, the present study focused on the role of these neurosteroids in nociception at the spinal level. In order to limit the effect of DHEAS/DHEA to the spinal cord, in this study we used i.t. administration of these neurosteroids. This was accomplished using the following experimental design. First, behavioural tests were performed to determine the effects of i.t. injection of DHEAS or DHEA on mechanical pain threshold. Then the mechanism of action of these neurosteroids was determined by investigating the possible involvement of sigma-1 or GABA$_A$ receptors. Finally, this hypothesis was further supported by administration of another neurosteroid, progesterone, which acts as an endogenous blocker of both sigma-1 and GABA$_A$ receptors (Tsutsui *et al.*, 2000; Ueda *et al.*, 2001; Bicikova and Hampl, 2007).

Methods

Animals

Male ICR mice (24–30 g) were purchased from the Laboratory Animal Center of Seoul National University (Seoul, South Korea). They were free to access food and water and maintained in temperature- and light-controlled rooms (23 ± 2°C, 12/12 h light/dark cycle with lights on at 08h00min) for at least 1 week prior to beginning an experiment. The experimental protocols for animal usage were reviewed and approved by the SNU Animal Care and Use Committee and conform to NIH guidelines (NIH publication No. 86-23, revised 1985).

Drugs

The following drugs were used: DHEA, DHEAS, muscimol (a GABA$_A$ receptor agonist), bicuculline (a GABA$_A$ receptor antagonist), BD-1047 {N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino) ethylamine dihydrobromide; a sigma-1 receptor antagonist} and progesterone. BD-1047 and muscimol were purchased from Tocris (Bristol, UK). The remaining drugs were obtained from Sigma Chemical Company (St. Louis, MO, USA). DHEA and progesterone were diluted in 1% DMSO (dimethyl sulphoxide) in corn oil. The vehicle for DHEAS and bicuculline was 1% DMSO in saline. The remaining drugs were diluted in physiological saline. The

drug and molecular target nomenclature used in this article conforms to the *British Journal of Pharmacology*'s 'Guide to receptors and channels' (Alexander *et al.*, 2008).

Intrathecal drug administration

Drugs were dissolved in 5 μL of vehicle. We injected a 5 μL volume intrathecally because data suggest that this is likely to be the upper limit that can be reliably injected into a mouse without any appreciable redistribution of the drug through the cerebrospinal fluid to the basal cisterns of the brain (Rieselbach *et al.*, 1962). Intrathecal injections were made into the L5-L6 intervertebral space of unanaesthetized mice using a 10 μL Hamilton syringe connected to a 30-gauge needle as previously described by Hylden and Wilcox (1980). The flick of the tail was considered indicative of a successful i.t. administration. The control group received an i.t. injection of vehicle.

Mechanical allodynia test

The von Frey monofilaments (Stoelting, Wood Dale, IL, USA) were used to quantify mechanical allodynia. On each testing day, mice ($n = 7$–8) were habituated inside a small plastic chamber (12 cm wide × 11 cm long × 15 cm high) with a floor of soft wire mesh for 1 h. Mechanical sensitivity was tested with von Frey hairs with bending forces from 0.07–6 g. The 50% mechanical withdrawal threshold (the force of the von Frey hair in g to which an animal reacts in 50% of the presentations) was determined on the basis of the Dixon up-and-down method (Dixon, 1980; Chaplan *et al.*, 1994) modified for mice (Sommer and Schafers, 1998). Testing was initiated with the 0.6 g hair. The von Frey filaments were applied from underneath the grid floor perpendicular to the plantar surface until slight buckling occurred. They were held for a period of approximately 3 s before being removed. A positive response was recorded if the paw was withdrawn. Ambulation was considered an ambiguous response, and in such cases the stimulation was repeated. In the event of a positive response, the next weaker filament was then applied and the next measurement recorded. In the absence of a response, then the next stronger filament was presented. This consecutive way of applying filaments was continued until six responses in the immediate vicinity of the 50% threshold were obtained. The resulting sequence of positive and negative responses was used to interpolate the 50% withdrawal threshold. Data are presented as % of the baseline value.

Statistical analysis

All values are expressed as the mean ± SEM. Statistical analysis was performed using Prism 4.0 (Graph Pad Software, San Diego, USA). The area under the curve was analysed using ANOVA followed by a Newman–Keuls *post hoc* test. For analysis of nociceptive sensitivity to mechanical stimulation at the different time points and under different treatment conditions, the data were analysed using a two-way repeated-measures ANOVA followed by a *post hoc* Bonferroni analysis. Differences with $P < 0.05$ were considered significant.

Results

The effect of i.t. DHEAS on nociceptive threshold

The mean value for the 50% nociceptive threshold for mechanical stimulation using the up–down method was 1.32 ± 0.25 g before treatment. The baseline values obtained for each treatment group were not statistically different. However, i.t. injection of DHEAS (150, 300 or 600 pmol) dose-dependently decreased withdrawal threshold to mechanical stimulation compared with that of the vehicle control group (Figure 1A and B). In particular, the mechanical allodynia induced by the 300 and 600 pmol doses of DHEAS peaked during the 10–60 min time period following i.t. injection of DHEAS and then returned to baseline by 180 min post
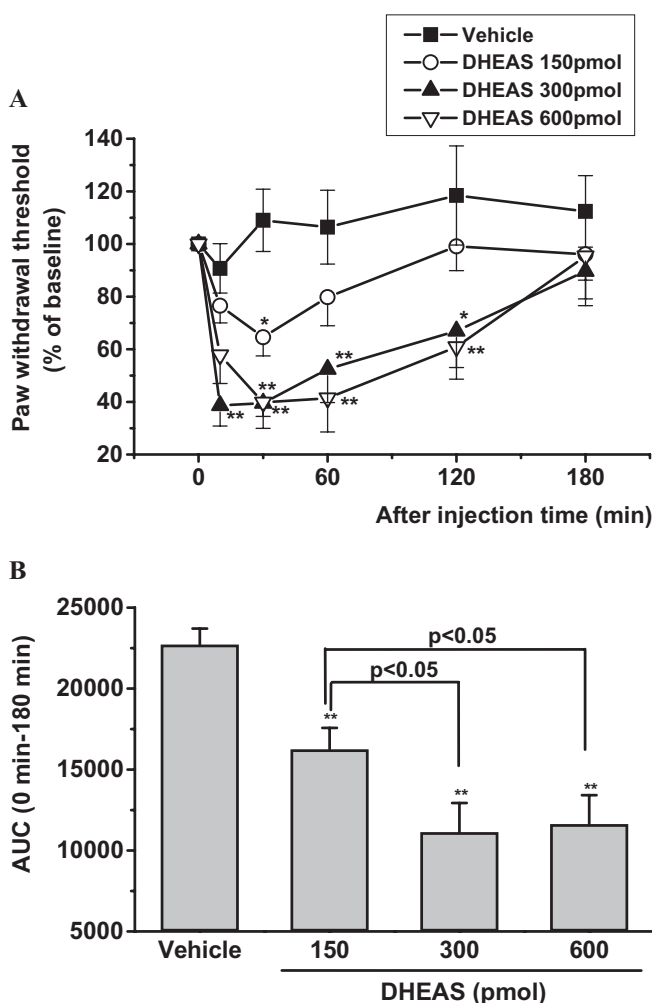
Figure 1 (A) The effect of intrathecal administration of three different doses of dehydroepiandrosterone sulphate (DHEAS) (150, 300 and 600 pmol per mouse) on the nociceptive threshold to mechanical stimulation. Data are presented as the percentage of pre-baseline value. **$P < 0.01$, *$P < 0.05$: significantly different from the value of the vehicle group (dimethyl sulphoxide 1% in saline) at same time point. (B) The mean area under the curve for paw withdrawal threshold for the vehicle-treated group and for each of the three groups receiving different doses of DHEAS for the period from 0 to 120 min after injection. **$P < 0.01$, different from the value of the vehicle group; vertical lines show SEM, $n = 8$ mice per group. AUC, area under the curve.

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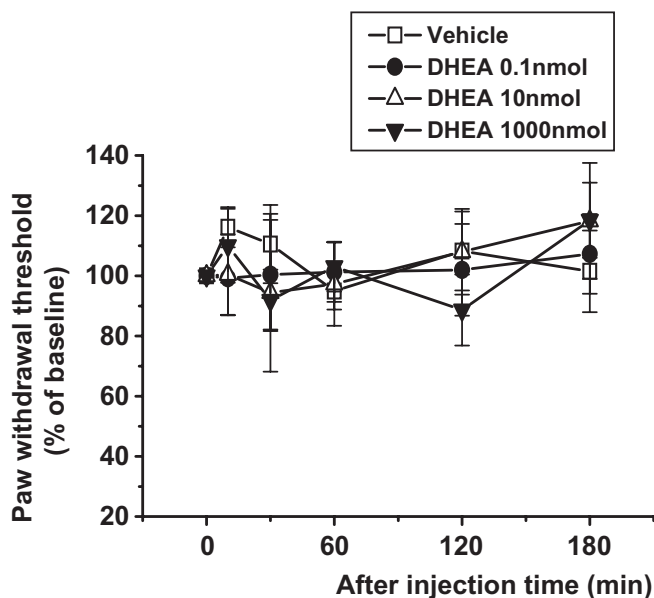
Figure 2 The effect of intrathecal administration of three different doses of dehydroepiandrosterone (DHEA) (0.1, 10 and 1000 nmol per mouse) on the nociceptive threshold to mechanical stimulation. The vehicle control group was treated with 1% dimethyl sulphoxide in corn oil. Data are presented as a percentage of pre-baseline values; *n* = 8 mice per group.

injection (Figure 1A). Conversely, i.t. injection of DHEA had no effect on the mechanical nociceptive threshold, even at the highest dose given (1 μmol, Figure 2).

The role of sigma-1 receptor or GABA$_A$ receptor on DHEAS-induced pain facilitation

The 300 pmol dose of DHEAS was selected for these experiments because this dose showed a maximum mechanical allodynia response. The development of DHEAS-induced mechanical allodynia was dose-dependently reversed by pretreatment with the sigma-1 receptor antagonist, BD-1047 (Figure 3A) or by pretreatment with the GABA$_A$ receptor agonist, muscimol (Figure 3B). Neither administration of BD-1047 (100 nmol) nor muscimol (0.3 nmol) alone modified the mechanical nociceptive threshold (data not shown).

The effect of i.t. DHEA or DHEA plus bicuculline on pain sensation

We first examined whether administration of different doses of the GABA$_A$ receptor inhibitor, bicuculline, had an effect on the normal nociceptive threshold. We found that i.t. injection of bicuculline induced mechanical allodynia in a dose-dependent fashion as shown in Figure 4A. Interestingly, although the 30 pmol dose of bicuculline alone had no effect on the mechanical nociceptive threshold (Figure 4A and B), when this dose of bicuculline was combined with DHEA (150, 300 or 600 pmol), it dose-dependently produced mechanical allodynia (Figure 4B). Moreover, the mechanical allodynia induced by DHEA (300 pmol) + bicuculline was reversed by pretreatment with BD-1047 or muscimol (Figure 4C).
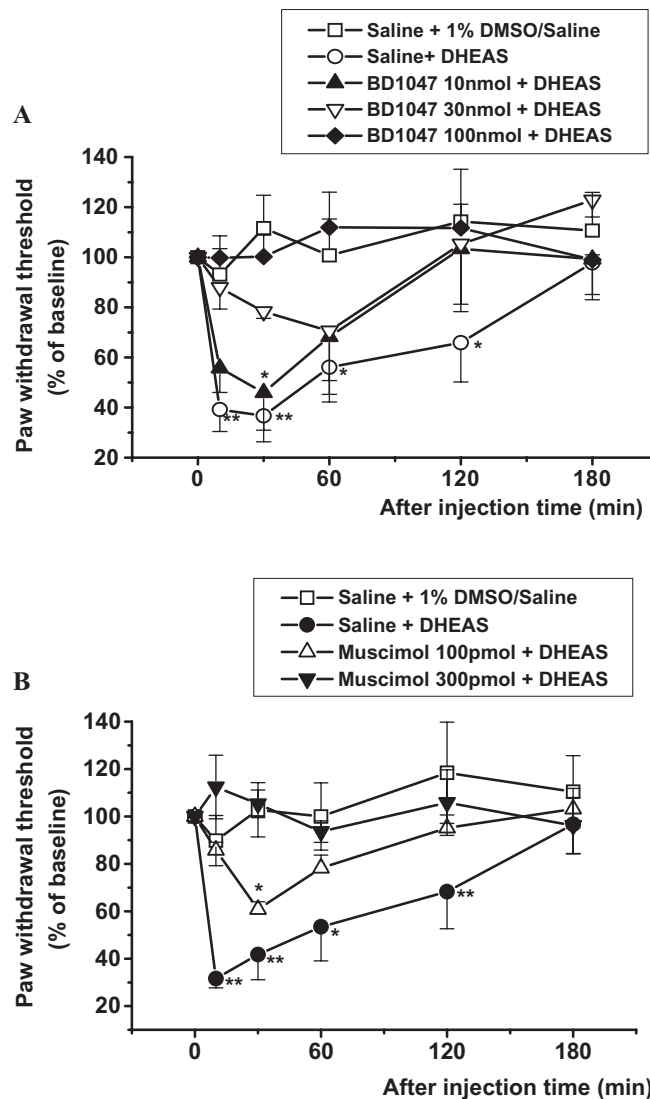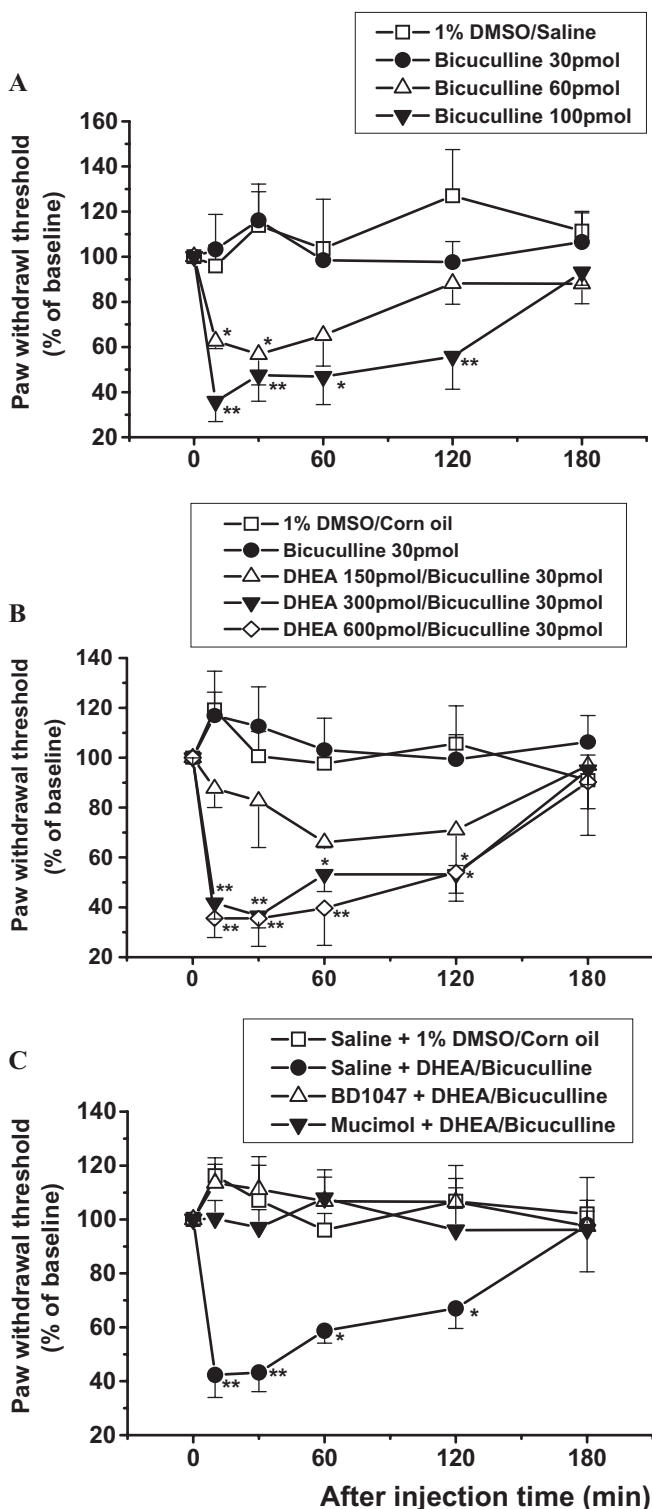

Figure 3 The effect of intrathecal dehydroepiandrosterone sulphate (DHEAS) (300 pmol per mouse) administered 5 min after i.t. pretreatment with BD-1047 (A) or muscimol (B) on the nociceptive threshold to mechanical stimulation. Data are presented as a percentage of the pre-baseline values. **$P < 0.01$, *$P < 0.05$: different from the vehicle control group [saline + 1% dimethyl sulphoxide (DMSO) in saline] value; *n* = 7 mice per group.

The role of progesterone in the mechanical allodynia induced by DHEAS or DHEA plus bicuculline
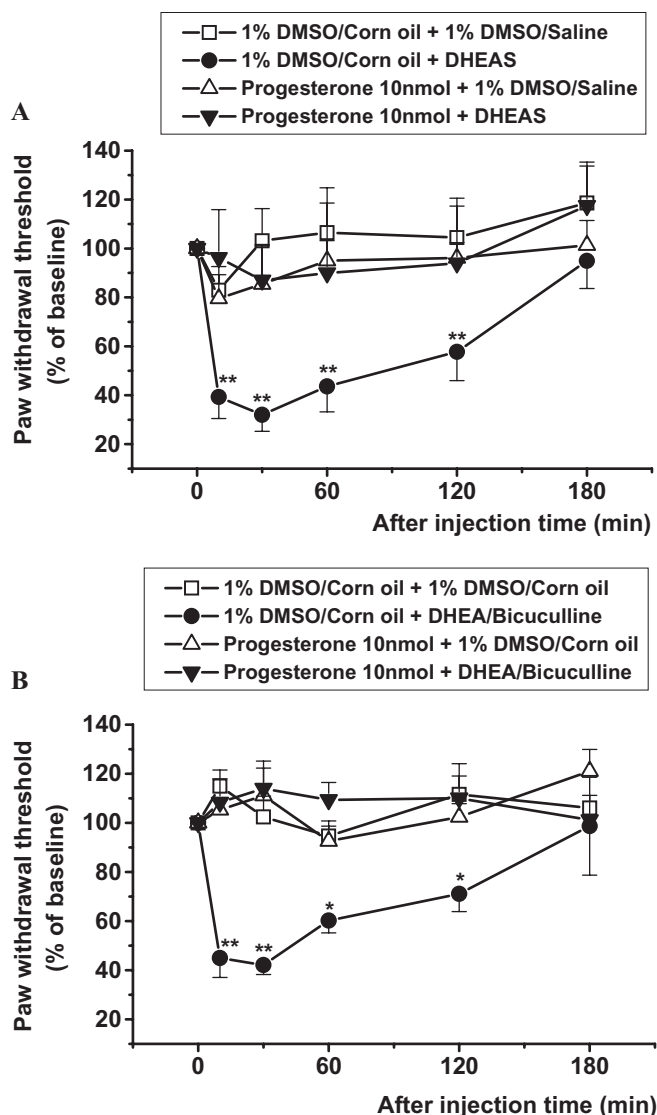
Intrathecal pretreatment with progesterone completely blocked DHEAS (300 pmol)-induced mechanical allodynia (Figure 5A). Moreover, i.t. pretreatment with progesterone also blocked the mechanical allodynia produced by DHEA (300 pmol) combined with bicuculline (30 pmol; Figure 5B). Conversely, i.t. progesterone (10 nmol) treatment alone had no effect on nociceptive thresholds to mechanical stimulation.

Discussion

The present study demonstrated that i.t. injection of DHEAS in mice enhanced the animal's sensitivity to mechanical

Figure 4 (A) The effect of intrathecal injection of bicuculline on the paw withdrawal threshold to mechanical stimulation. (B) The effect of a combination of dehydroepiandrosterone (DHEA) and bicuculline (DHEA/bicuculline) on the paw withdrawal threshold to mechanical stimulation. (C) The effect of intrathecal pretreatment with BD-1047 (100 nmol) or muscimol (0.3 nmol) on mechanical allodynia induced by DHEA + bicuculline. Data are presented as the percentage of pre-baseline values. **$P < 0.01$, *$P < 0.05$: different from vehicle control group [(A) 1% dimethyl sulphoxide (DMSO) in saline; (B) 1% DMSO in corn oil; (C) saline + 1% DMSO in corn oil] value; $n = 7$ mice per group.

Figure 5 The effect of intrathecal dehydroepiandrosterone sulphate (DHEAS) (300 pmol per mouse) (A) or a combination of DHEA plus bicuculline (B) given 5 min after intrathecal pretreatment with progesterone on the nociceptive threshold for mechanical stimulation. Data are presented as the percentage of pre-baseline values. **$P < 0.01$, *$P < 0.05$: different from vehicle control group [(A) 1% dimethyl sulphoxide (DMSO) in corn oil + 1% DMSO in saline, (B) 1% DMSO in corn oil + 1% DMSO in corn oil] value; $n = 7$ mice per group.

stimuli and that this resulting mechanical allodynia was associated with the effect of DHEAS on sigma-1 and GABA$_A$ receptors. Previous studies have shown that alterations in the levels of DHEA or DHEAS in the brain can allosterically modulate GABA$_A$, N-methyl-D-aspartate and sigma-1 receptors (McEwen, 1991; Genud *et al.*, 2009). With respect to sigma-1 receptors, recent studies from our laboratories have demonstrated that i.t administration of sigma-1 agonists significantly enhance peripheral nociceptive responses, suggesting that spinal sigma-1 receptors play an important role in nociceptive processing (Kim *et al.*, 2006; 2008; Roh *et al.*, 2008a,b) Moreover, the results of the present study indicate that sigma-1 receptors also play an important role in

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DHEAS-induced mechanical allodynia. On the other hand, fast inhibitory controls, which act at the spinal cord level and are mediated by GABA_A receptors, appear to play an important role in preventing or reducing the development of mechanical allodynia in a number of different pathological pain states (Galluzzi, 2007). Indeed, data obtained in both the present study and previous research studies have demonstrated that effective blockade of spinal GABA_A receptors by i.t. bicuculline produces mechanical allodynia (Onaka *et al.*, 1996). The present study extends these findings by indicating that DHEAS-induced mechanical allodynia is also mediated by negative modulation of spinal GABA_A receptors.

In contrast to DHEAS, i.t. injection of non-sulphated DHEA failed to produce a pain facilitatory effect on mechanical stimulation, even at the highest dose (1 μmol) tested in this study. One plausible explanation for this discrepancy stems from that fact that there are multiple recognition sites associated with GABA_A receptors, including barbiturate, GABA and benzodiazepine-type ligand-binding sites. In this regard, Park-Chung and his colleagues (Park-Chung *et al.*, 1999) demonstrated that sulphated neurosteroids, such as DHEAS and allopregnanolone sulphate, act at distinct sites on the GABA_A receptor in comparison with their unsulphated form. In support of this finding, DHEA does not inhibit the binding of DHEAS to brain membranes, suggesting that they may also bind to distinct sites (Demirgoren *et al.*, 1991). DHEAS inhibits the binding of [^{3}H]-GABA to both cerebral cortex and cerebellar membranes, while DHEA (up to 100 μmol·L^{-1}) has no effect on GABA binding to these brain regions (Sousa and Ticku, 1997). Similarly, the affinity of DHEA to GABA-binding sites (using a [^{3}H]-muscimol assay) was too low to detect in rat cerebral cortex, while the affinity of DHEAS was more prominent (IC$_{50}$ = 557 μmol·L^{-1}, Mehta and Ticku, 2001). Taken together, DHEA has very low affinity for the GABA/muscimol binding site on GABA_A receptors and this characteristic may produce the pharmacological differences observed when comparing DHEA with DHEAS. In support of this assumption, we have also demonstrated that i.t. administration of a combination of DHEA and a sub-effective dose (30 pmol) of bicuculline, a GABA_A receptor antagonist, significantly decreased the nociceptive thresholds to mechanical stimulation.

Interestingly, Kibaly *et al.* (2007) have shown that i.t. DHEA administration induces mechanical and thermal hyperalgesia in naïve rats and this effect is totally blocked by administration of a sigma-1 receptor antagonist. Although DHEA is less potent at inhibiting GABA_A receptors, it is still a sigma-1 activator. Therefore, a high dose of DHEA could induce pain facilitation. In this regard, it is important to note that the dose of DHEA used in the study by Kibaly *et al.* (2007) was significantly higher (approximately 10 μmol) than that used in the present study. Thus, the discrepancy between the present data and those of Kibaly *et al.* (2007) may relate to the dose of DHEA used or to possible species differences between mice and rats.

The present results suggest that the allodynia effects of DHEAS or DHEA are mediated through both sigma-1 and GABA_A receptors in a possible synergistic, but not additive way. Supporting this assumption, the allodynia effects of DHEAS were completely blocked by administration of either a sigma-1 receptor antagonist or a GABA_A agonist. Moreover,

while DHEA did not induce allodynia, even at the highest dose (1000 nmol) tested, a very low dose (0.3 nmol) of DHEA induced allodynia when it was combined with a low dose of bicuculline, which by itself did not induce allodynia. Importantly, this pain facilitatory effect of DHEA plus bicuculline was completely blocked by administration of either a sigma-1 antagonist or a GABA_A receptor agonist. A possible synergistic interaction between sigma-1 and GABA_A receptors has not been convincingly described to date. However, Mtchedlishvili and Kapur (2003) reported that low concentrations of pregnenolone sulphate, which also acts as negative modulator of GABA_A receptors, inhibited presynaptic GABA release in cultured hippocampal neurons. This effect was also abolished by the sigma-1 receptor antagonist, suggesting that sigma-1 receptor activation modulates GABA release and neurotransmission. As this observation does not provide a mechanistic picture of how the GABA_A and the sigma-1 receptors interact, nor any insight into whether this interaction is direct or indirect, it is clear that further work is required to determine the exact mechanisms by which such synergistic interaction occurs in the spinal cord resulting in decreased mechanical nociceptive sensitivity.

Under chronic pain conditions, such as the neuropathic pain that often develops as a result of peripheral nerve injury, the effect of these neurosteroids on spinal pain transmission may in fact be altered. Our laboratory has recently reported that sigma-1 receptor expression significantly increases in the ipsilateral spinal cord dorsal horn from day 1 to day 3 post surgery in a rat chronic constriction injury model of neuropathic pain (Roh *et al.*, 2008b). Moreover, following peripheral nerve injury, there is an apparent loss of GABAergic inhibitory interneurons in the dorsal horn of the spinal cord (Zeilhofer, 2008). Collectively, these findings raise the possibility that changes in sigma-1 receptor expression and alterations in GABAergic transmission may play important roles in the development of central sensitization of pathological pain states, such as neuropathic pain. In support of this assumption, it has demonstrated that i.t. injection of the sigma-1 receptor antagonist, BD-1047, significantly blocks the induction of mechanical allodynia in rats with neuropathic pain induced by chronic constriction injury (Roh *et al.*, 2008b). Conversely, BD-1047 administered alone does not show any pain-inhibitory effect. Additionally, GABA_A receptors appear to play an important role in neuropathic pain, as it has been demonstrated that the GABA_A receptor agonist, isoguvacine (Malan *et al.*, 2002), reverses both the tactile allodynia and thermal hyperalgesia produced by spinal nerve ligation. In naïve animals, however, i.t. administration of isoguvacine does not cause any anti-nociceptive effects as measured by the tail-flick test. Moreover, i.t. treatment with another GABA_A agonist, muscimol, also resulted in a dose-dependent antagonism of the allodynia associated with a rodent neuropathic pain model (Hwang and Yaksh, 1997). Collectively, these data together with the current findings suggest that co-administration of a sigma-1 agonist with a GABA_A receptor antagonist would have a greater therapeutic effect on neuropathic pain than administration of either drug alone. In addition, under pathological pain conditions, the effect of DHEAS or DHEA on spinal pain transmission may be altered. Thus, the combination of an increase in spinal sigma-1 receptor

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expression and the loss of GABAergic inhibitory interneurons may induce an augmentation in the pain facilitatory effect of DHEAS in pathological pain states compared with its spinal cord effects in normal animals or humans. Further study is required to establish the actual relationship between DHEA and DHEAS and the development or maintenance of chronic pain, as well as the potential interaction of these neurosteroids with GABA$_A$ receptors and sigma-1 receptors in patients with chronic pain.

Progesterone and its metabolites have also been proposed to act as an endogenous sigma-1 receptor antagonist as well as a potent positive allosteric modulator of the GABA$_A$ receptor (Maurice *et al.*, 2006). Therefore, it is not surprising that we found that pretreatment with progesterone completely blocked the mechanical allodynia induced by DHEAS and DHEA plus bicuculline. This suggests that spinal nociceptive transmission can be positively or negatively regulated by endogenous neurosteroids.

In conclusion, in the present study we observed that i.t. injection of the neurosteroid, DHEAS, produced mechanical allodynia and that this effect was mediated by activation of sigma-1 receptors and inhibition of GABA$_A$ receptors. Although i.t. injection of DHEA alone or of a sub-effective dose of the GABA$_A$ receptor antagonist, bicuculline, alone did not alter mechanical nociceptive thresholds, injection of a combination of DHEA and bicuculline induced mechanical allodynia similar to that induced by DHEAS alone. This suggests that DHEA is pharmacologically much less potent at inhibiting GABA$_A$ receptors than DHEAS. These results also indicate that i.t. DHEAS produces a significantly greater pain facilitatory effect than DHEA. Moreover, i.t. pretreatment with the neurosteroid, progesterone, reversed the DHEAS-induced mechanical allodynia, indicating that endogenous neurosteroids can have both nociceptive and anti-nociceptive effects. Because neurosteroids are synthesized in the dorsal horn of the spinal cord and have limited side effects, this study opens interesting possibilities for further investigations into the mechanisms underlying neurosteroid modulation of spinal pain transmission and more importantly for potential therapeutic use of exogenous neurosteroids to treat pathological pain conditions.

Acknowledgements

Conflicts of interest

None.

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