

Regulatory effect of dehydroepiandrosterone on spinal cord nociceptive function

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Biosynthesis of DHEA in the SC
 - 3.1. Evidence for the expression of cytochrome P450c17 in the SC
 - 3.2. Biological activity of P450c17 in the SC
 - 3.3. Hypothetic significance of DHEA production in the SC
4. Modification of DHEA production in the SC during neuropathic pain situation
5. Behavioral evidence for a regulatory effect of DHEA on spinal nociceptive pathways
6. Summary and perspective
7. Acknowledgements
8. References

1. ABSTRACT

To characterize endogenous molecules regulating nociception, various groups have focused on dehydroepiandrosterone (DHEA). Indeed, DHEA modulates NMDA and P2X receptors which control neurobiological activities including nociception. Thus, various results were published on DHEA ability to regulate nociception but the data were interpreted separately. To provide an overview, we analyzed here the current knowledge on DHEA regulatory action on the spinal cord (SC) which is pivotal for nociception. DHEA endogenously synthesized in the SC appears as a key factor regulating nociception. However, DHEA effects on nociceptive mechanisms are complex. Acute DHEA treatment exerts a biphasic effects on nociception (a rapid pro-nociceptive action and a delayed anti-nociceptive effect). Chronic DHEA treatment increased basal nociceptive thresholds in neuropathic and control rats, suggesting that androgenic metabolites of DHEA exerted analgesic effects while DHEA itself caused a rapid pro-nociceptive action. To get more insights into DHEA effects on nociception, we provided a hypothetical scheme recapitulating cellular mechanisms of action of DHEA in the control of nociception. Perspective is opened for the development of DHEA-based strategies against pathological pain.

2. INTRODUCTION

The anatomical nociceptive circuit implies peripheral nociceptors which are essential for the perception of pain. These terminals belong to primary sensory neurons whose cell bodies are located in dorsal root ganglia. Central axons of primary afferents terminate in the spinal cord (SC) dorsal horn (DH) and second-order spinal neurons projecting to the brain often have convergent inputs from different sensory fibers and tissues. Therefore, the SC appears as a pivotal structure in nociception and pain transmission. While the spinal anatomical circuit involved in nociception is well identified, molecular and neurochemical components of pain modulation deserve further clarification even though substantial progress has been made over the two last decades (1-11). It is most probable that nociception and pain, especially pathological pains, are characterized by multiple or sophisticated cellular mechanisms so that the identification of all of the endogenous factors involved appears extremely important for the development of efficient therapeutic strategies (12-17). Most of nociceptive and pain processes already identified seem as subjected to an expression of neural plasticity or to the capacity of neurons to change their function, chemical profile and structure or to trigger apoptotic processes, particularly in

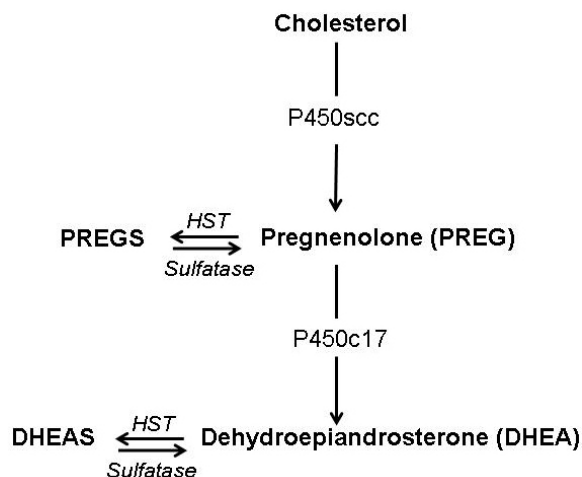


Figure 1. Biochemical pathways leading to DHEA biosynthesis. P450scc, cytochrome P450side-chain-cleavage; P450c17, cytochrome P450c17 or 17 α -hydroxylase/17,20lyase; HST, Hydroxysteroid sulfotransferase.

chronic pain states (18). Thus, preventing these neural modifications responsible for chronic pain is a real challenge for biomedical research. To take up this challenge, various neurotransmitters and endogenous compounds modulating neural activity and plasticity have been investigated to determine their effective role in the regulation of nociceptive mechanisms (4, 5). Because dehydroepiandrosterone (DHEA) is an endogenous molecule generally considered as a crucial modulator of several biological functions (for review, 19), DHEA has raised a particular interest for the regulation of nociception. Indeed, DHEA and its sulfate derivative (DHEAS) are the most abundant steroids secreted by the human adrenal gland and decreased DHEA blood levels are correlated with various age-related physiological deficiencies (19, 20). It has been demonstrated that, within nanomolar and micromolar concentration ranges, synthetic DHEA controls various mechanisms in the central nervous system (CNS) of rodents (21, 22). DHEA induces prominent increases in the numbers of neurons and astrocytes with extensions of the processes of both cell types (23). In particular, DHEA promotes axonal growth and morphological indices of synaptic contacts whereas DHEAS stimulates dendritic growth and branching in cultured embryonic neuronal cells (24). In addition, DHEA has been reported to increase neuronal excitability when directly applied to septal-preoptic neurons (25). Moreover, several studies have also indicated that DHEA and DHEAS modulate NMDA and P2X receptors which are involved in numerous activities of the nervous system including nociception and pain transmission (21, 22, 26). Consequently, various research groups have focused their efforts on DHEA in order to determine its ability or potential to regulate nociceptive and pain mechanisms (26-31). These groups published a variety of results that were interpreted separately so that an integrated or complete view of the situation is not available. Therefore, we have decided to recapitulate, to discuss and to highlight in the present paper the current knowledge on the regulatory action exerted by DHEA on the SC which plays a pivotal role in nociception and pain.

3. BIOSYNTHESIS OF DHEA IN THE SC

The biosynthesis of DHEA requires catalytic actions of two different cytochromes: P450 side-chain-cleavage (P450scc) which converts cholesterol into pregnenolone (PREG) that is transformed successively into 17-hydroxy-PREG and DHEA by a single microsomal enzyme, cytochrome P450c17 (P450c17) (Figure 1). Unlike in humans, plasma concentrations of DHEA/DHEAS are extremely low or undetectable in adult rodents (32, 33). Concurrently, P450c17 gene is expressed in human adrenals and gonads while in rodents, the enzyme is present in gonads but not in adrenals (34-38). By using synthetic DHEA and the rodent brain as model, pharmacological and behavioral studies suggested that DHEA may be a potent endogenous modulator of several neurobiological mechanisms and its decrease during ageing is correlated to various physiological deficits (19-21). However, the validity of such hypothesis remains speculative because the adult rodent endocrine glands do not secrete significant amounts of DHEA (32, 33). Thus, it appears that in adult rodents, DHEA could be a potent endogenous modulator of the CNS activity only if this steroid is synthesized within the nervous tissue. Consequently, the first step for the demonstration of a possible role of endogenous DHEA in the modulation of the SC nociceptive function was the investigation of local production of DHEA in spinal neural pathways.

3.1. Evidence for the expression of cytochrome P450c17 in the SC

Real time polymerase chain reaction after reverse transcription made it possible to detect significant amounts of specific mRNA encoding P450c17 in all segments of adult rat SC (29). In particular, the specificity of PCR products was confirmed by analyses based on melting temperature in the same closed capillary used for amplification, an approach which avoided the risk of contamination and enabled easy differentiation of specific fragments from non-specific products (39, 40). The normalisation of P450c17 product to GAPDH revealed that the concentrations of P450c17 mRNA present in the adult rat SC were sufficient to justify a substantial expression of the enzymatic protein in spinal tissue (29).

The availability of a specific antiserum against P450c17 allowed the assessment by western blot of the occurrence of P450c17 protein in the SC and testis. A specific P450 c17 protein was detected in total homogenates and microsomal fractions from the SC and testis using P450c17 antiserum which has also been utilized successfully to localize P450c17 in Leydig cells (29, 41, 42). The anti-P450c17 made it also possible to determine the anatomical and cellular distribution of P450c17-like immunoreactivity in the white and gray matters of the SC (29, 31). Double-labelling experiments with specific markers for neurons, astrocytes and oligodendrocytes (43-46) revealed that P450c17-immunostaining was expressed in both neurons and glial cells throughout the adult rat SC (29). However, in the white matter, P450c17 was mainly localized in astrocytes while the enzyme was detected in neurons and oligodendrocytes of the gray matter. In

Effect of DHEA on nociception

particular, an important population of neurons of the DH expressed P450c17-immunoreactivity suggesting that the enzyme may be involved in the modulation of sensory activity (4, 5, 47). Numerous neurons of the ventral horn also contained P450c17 indicating a possible correlation of the enzyme activity with motor function (48). Surprisingly, the P450c17-immunoreactive material was detected in the nucleoplasm of certain nerve cell bodies even though the enzyme is well known to be a microsomal protein in classical steroidogenic tissues (29, 42). It is possible that this apparent nuclear labelling corresponds to a localization of P450c17 in the endoplasmic reticulum associated to the nuclear membrane. Nevertheless, it is noteworthy that previous studies have also mentioned the nuclear localization of steroidogenic enzymes such as 17 β -hydroxysteroid dehydrogenase and 5 α -reductase isozymes in nuclear and cytoplasmic compartments of human and rat prostatic cells (49-52).

3.2. Biological activity of P450c17 in the SC

To demonstrate that P450c17-like immunoreactivity detected in the SC corresponds to an active form of the enzyme, pulse-chase experiments were performed to study the conversion of tritiated pregnenolone ($[^3\text{H}]\text{PREG}$) into radioactive metabolites by SC slices (29, 30). The results showed that $[^3\text{H}]\text{DHEA}$ was synthesized *de novo* from $[^3\text{H}]\text{PREG}$ in SC slices and the newly-produced steroid was characterized by the HPLC/Flo-One method (40, 53-56). In addition, among the newly-synthesized radioactive steroids from $[^3\text{H}]\text{PREG}$, two corresponded to $[^3\text{H}]\text{PROG}$ and $[^3\text{H}]\text{17-OH-PROG}$ indicating that the precursor ($[^3\text{H}]\text{PREG}$) was converted by 3 β -hydroxysteroid dehydrogenase (3 β -HSD) into $[^3\text{H}]\text{PROG}$ which was in turn transformed into $[^3\text{H}]\text{17-OH-PROG}$ by P450c17. In agreement with this observation, 3 β -HSD gene expression and activity have been shown in adult rat SC (57). Furthermore, the conversion of $[^3\text{H}]\text{PREG}$ into $[^3\text{H}]\text{DHEA}$ or $[^3\text{H}]\text{17-OH-PROG}$ was significantly reduced when the pulse-chase experiments were performed in the presence of ketoconazole, a selective inhibitor of P450c17 (58, 59). Moreover, kinetics studies showed that the absolute amount of $[^3\text{H}]\text{DHEA}$ synthesized from $[^3\text{H}]\text{PREG}$ reached a maximum within 3 h but declined slowly as only a 20% decrease was observed during the next 9 h (29). This result suggests that endogenous DHEA produced in the SC may be accumulated and reach sufficient or required concentrations to induce various neuroactive actions. The 20% decrease may correspond to the percentage of DHEA converted into DHEAS or into endogenous estradiol as observed in hippocampal neurons (60). DHEA can also be re-obtained from DHEAS by the activity of steroid sulfatase the presence of which has been shown in human and rodent nervous systems (61, 62).

3.3. Hypothetic significance of DHEA production in the SC

It has been demonstrated that DHEA promotes recovery of motor behavior after contusive SC injury in adult rodent (63). This observation was made by treating the injured SC with synthetic DHEA. Therefore, it is possible that local production of DHEA in the SC may

belong to endogenous mechanisms activated in the spinal neural tissue to cope with aggressive or traumatic situations. In support of this suggestion, in the white matter, P450c17-immunoreactivity was mainly expressed in astrocytes, a cell-type strongly involved in reactive gliosis characterizing the spinal neural tissue under traumatic states (64). It has also been reported that DHEA, PREG and sex steroids may affect brain repair by down-regulating gliotic tissue (65). Moreover, implication of DHEA was evidenced in the regulation of astroglia reaction to denervation of olfactory glomeruli (66). Collectively, these data indicate that DHEA endogenously synthesized in the CNS may be important for the control of neural plasticity. Various pharmacological studies have also suggested the involvement of synthetic DHEA/DHEAS in the modulation of sensory processes. A pro-nociceptive effect of DHEAS has been described in mice using peripheral flexor response test (27, 28). Electrophysiological data also revealed that DHEA potentiates native ionotropic ATP receptors containing the P2X2 subunit in rat sensory neurons (26). A competitive inhibition of the capsaicin receptor-mediated current by DHEA has recently been shown on dorsal root sensory neurons (67). Altogether, these studies suggest a potentially pivotal role for endogenous DHEA in the control of nociceptive transmission. This hypothesis is strongly supported by the localisation of P450c17 in numerous neurons of the DH [an important structure involved in nociception (4, 5)] and also by the local synthesis of DHEA in spinal neuronal networks. However, these data which were generally obtained in naive animals were not sufficient to prove that endogenous DHEA effectively modulate the SC nociceptive function and pain sensation. Therefore, additional series of investigations using the rat experimental model of neuropathic pain (68) were required to definitively confirm the regulatory role of endogenous DHEA on nociception.

4. MODIFICATION OF DHEA PRODUCTION IN THE SC DURING NEUROPATHIC PAIN SITUATION

Molecular experiments using quantitative real time polymerase chain reaction after reverse transcription revealed that the transcripts encoding P450c17 were down-regulated in the SC dorsal horn of rats subjected to peripheral neuropathic pain (30, 31). The down-regulation of P450c17 gene expression was accompanied by a marked decrease in P450c17 enzymatic activity in the SC as revealed by *in vitro* and *in vivo* biochemical experiments. Therefore, it appeared that the local synthesis of DHEA by sensory neural networks of the SC (29) was dramatically reduced under a chronic *pain situation*. To understand the reason why DHEA synthesis decreased in the SC of neuropathic pain rats, various series of behavioral and pharmacological studies were performed using exogenous DHEA and ketoconazole, a pharmacological P450c17 inhibitor that blocks DHEA formation allowing therefore the identification of the role played by DHEA endogenously produced by spinal nerve cells (69, 70).

5. BEHAVIORAL EVIDENCE FOR A REGULATORY EFFECT OF DHEA ON SPINAL NOCICEPTIVE PATHWAYS

A first series of behavioral experiments investigated the effects of acute subcutaneous administration of DHEA on nociceptive thresholds of naive rats (30). Injected concentrations in rats were determined on the basis of DHEA doses generally used in humans (26, 71-74). All behavioral measurements within 1.5 h interval following acute subcutaneous administration revealed that each tested dose of DHEA was capable of decreasing both thermal and mechanical nociceptive thresholds (30). These observations indicate that the rapid action of DHEA on nociception is a pro-nociceptive effect. In support of this suggestion, additional series of behavioral studies after acute intrathecal injections also showed that DHEA produced a rapid pro-nociceptive effect in naive rats and transiently potentiated the thermal hyperalgesia and mechanical allodynia characterizing neuropathic animals (30, 31). The pro-nociceptive effect of acute DHEA treatment was very strong when DHEA was directly applied to the lumbar SC. Indeed, acute intrathecal injection of 10 mg/kg of DHEA (a dose 7.5- or 15-fold lower than those used for subcutaneous administration) was capable of producing a nociceptive threshold decrease similar to that obtained with acute s.c. injections of DHEA at 75 mg/kg or 150 mg/kg (30). This result indicates that the SC sensory networks are strongly involved in the mediation of DHEA action on nociception. In addition, inhibition of the local synthesis of DHEA in spinal neural pathways by intrathecal administration of ketoconazole, a pharmacological P450c17 blocker (69, 70), produced analgesia in neuropathic rats and a potent anti-nociceptive effect in controls, demonstrating that DHEA produced by the SC (29) is an endogenous pro-nociceptive steroid (30). Therefore, it is possible to speculate that the down-regulation of P450c17 gene expression and DHEA formation in the SC may be an endogenous mechanism triggered by these animals to cope with the chronic pain condition. In support of this suggestion, suppression of DHEA synthesis in the SC by intrathecal injection of ketoconazole resulted in a significant analgesic effect that completely abolished in neuropathic rats the thermal hyperalgesia and mechanical allodynia evoked by sciatic nerve ligature (30, 31). Interestingly, previous studies showed that neuropathic painful state also produced in SC an up-regulation of the biosynthetic pathway of neurosteroid allopregnanolone, a potent allosteric activator of GABA_A receptors (40). Thus, the process of neurosteroid biosynthesis appears to be a mechanism selectively regulated in SC sensory networks during neuropathic pain to increase, on the one hand, the production of anti-nociceptive neurosteroids such as allopregnanolone and tetrahydrodeoxycorticosterone (75, 76) and to reduce, on the other hand, the formation of pro-nociceptive neurosteroids such as DHEA. Investigations of interactions between major neurotransmitters involved in pain transmission and P450c17 activity in the SC may certainly help to

elucidate in the future the mechanisms underlying the inhibitory impact of neuropathic pain on DHEA biosynthesis in the SC. The data also revealed that, contrary to the rapid pro-nociceptive effect exerted by DHEA itself (before being metabolized), androgenic metabolites deriving from DHEA may induce a delayed analgesic or anti-nociceptive action in neuropathic pain or control rats, respectively (29-31). Indeed, the pro-nociceptive effect of acute DHEA treatment was followed by a delayed increase of the thermal and mechanical thresholds (30). In addition, chronic administration of DHEA, which is well-known to generate a permanently high level of androgens in the blood, significantly increased and maintained elevated the basal nociceptive thresholds in neuropathic and control rats. In particular, after one week of chronic DHEA treatment, the rapid pro-nociceptive effect evoked by DHEA became undetectable when time-course behavioral analyses were performed within the 4 h interval following the injection while the delayed anti-nociceptive action persisted (30). Moreover, intrathecal administration of testosterone, one of the major androgens deriving from DHEA (77), induced a significant analgesic effect in neuropathic rats by increasing nociceptive thresholds on the ipsilateral and contralateral paws (30, 31). In agreement with these results, previous investigations have reported androgen-induced analgesic effects and discussed the possible mechanisms of action of testosterone and its 5 α -reduced metabolites in pain modulation (78, 79). However, the rapid pro-nociceptive action exerted by DHEA itself before being metabolized, as well as the occurrence of a biphasic effect of acute DHEA treatment had never been described. Therefore, in order to clarify as much as possible these findings, pharmacological analyses were performed to provide valuable clues on the mechanism of action underlying the rapid pro-nociceptive effect of acute DHEA treatment (30). In fact, until now, a specific receptor for DHEA has not been characterized. DHEA acts as an allosteric modulator of NMDA and P2X receptors that play a pivotal role in the control of nociceptive transmission (4, 26, 80, 81). Thus, the pro-nociceptive effect of DHEA may be explained by DHEA action on NMDA or P2X receptors localized in the SC dorsal horn (82-84). In particular, it has been demonstrated that DHEA activates the glutamatergic transmission by potentiating NMDA responses via sigma type 1 receptor or S1-R (85). Indeed, several studies which identified functional interactions between S1-R and NMDA receptors revealed that DHEA triggers through S1-R intracellular cascades leading to phosphorylation of NMDA receptors (85-88). Therefore, based on the crucial role played by the glutamatergic system in nociception (4), it has been determined whether the mechanism of action of DHEA on pain modulation involves the process of S1-R-evoked NMDA receptor activation (30). The data clearly demonstrated that BD1047, a selective antagonist of S1-R (89), completely blocks the rapid pro-nociceptive effect of DHEA on thermal and mechanical pain thresholds (30). Since the presence of S1-R has been well-established in SC sensory networks where DHEA is locally synthesized by P450c17-

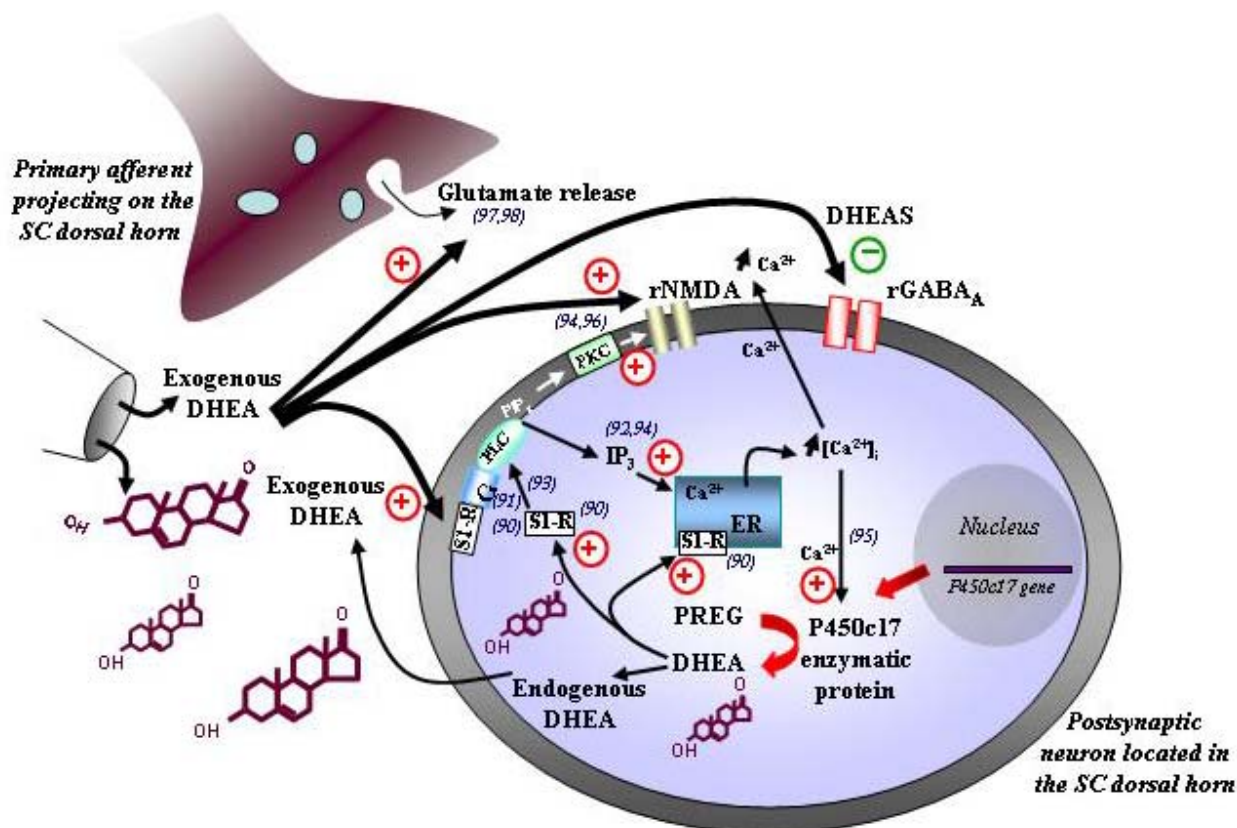


Figure 2. Hypothetical scheme recapitulating cellular mechanisms of action of DHEA in the regulation of nociception. Acting through an allosteric site, endogenous or exogenous DHEA may directly modulate the action of glutamate (released by primary afferents) on NMDA receptors (rNMDA) expressed by SC dorsal horn neurons. DHEA may also interact indirectly with rNMDA by triggering from sigma-1 receptor (S1-R) a phospholipase C (PLC)-dependent signalling leading to rNMDA modulation. Another possibility is the conversion of DHEA into DHEAS which is able to modulate in a dose-dependent manner GABA_A receptors (r GABA_A). ER, endoplasmic reticulum.

positive cells (29, 90), it appears that endogenous DHEA may control spinal nociceptive mechanisms through paracrine or autocrine modulation of the process of S1-R-induced NMDA receptor activation.

6. SUMMARY AND PERSPECTIVE

The present review shows that endogenous DHEA is a key factor involved in the regulation of SC nociceptive function. The paper also highlighted the fact that DHEA effects on nociceptive mechanisms are complex. Acute treatment with exogenous DHEA exerts a biphasic effects on nociception, that is, a rapid pro-nociceptive action and a delayed anti-nociceptive effect. Chronic DHEA treatment increased and maintained elevated basal nociceptive thresholds in neuropathic and control rats, suggesting that androgenic metabolites generated from daily administered DHEA exerted analgesic effects while DHEA itself caused a rapid pro-nociceptive action. To shed more light on the current knowledge on DHEA effects on nociception, we provide a hypothetical scheme recapitulating cellular mechanisms of action of DHEA in the regulation of nociception and pain (Figure 2). Taken together, these data

open interesting perspective for the development of efficient DHEA-based strategies against pathological pain.

7. ACKNOWLEDGEMENTS

This work was supported by grants from Universite de Strasbourg, France. C.K. was a recipient of a fellowship from Ministere de l'Education Nationale et de la Recherche. L.M. is a postdoctoral fellow supported by Association Titoine (Normandie, France).

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Effect of DHEA on nociception

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Effect of DHEA on nociception

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Effect of DHEA on nociception

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Effect of DHEA on nociception

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Key Words : Neuroactive steroid, Neurosteroid, Pain, Sensory system, Steroid, Nervous System, Review

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