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Competitive Inhibition of the Capsaicin Receptor-Mediated Current by Dehydroepiandrosterone in Rat Dorsal Root Ganglion Neurons

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Running title: Inhibition of the Capsaicin Receptor by DHEA

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ABBREVIATION: DHEA, 5-androsten-3β-ol-17-one (dehydroepiandrosterone); 3α-DHEA, 5-androsten-3α-ol-17-one; DHEAS, 5-androsten-3β-ol-17-one sulfate (dehydroepiandrosterone sulfate); PS, pregnenolone sulfate; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NMDA, N-methyl-D-aspartate; GABA, γ-aminobutyric acid; PP, protein phosphatase

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ABSTRACT

The effects of dehydroepiandrosterone (5-androsten-3β-ol-17-one; DHEA) and related steroids on the capsaicin receptor-mediated current were studied in acutely dissociated rat dorsal root ganglion neurons using the whole-cell voltage-clamp technique. DHEA rapidly and reversibly inhibited the capsaicin-induced current in a concentration-dependent manner, with an EC_{50} of 6.7 μM and a maximal inhibition of 100%. DHEA increased the capsaicin EC_{50} with little effect on the capsaicin maximal response, suggesting that the blocking action of DHEA is competitive. Neither the capsaicin response nor inhibition of the capsaicin response by extracellularly applied DHEA was significantly affected by inclusion of a saturating concentration of DHEA in the electrode buffer, arguing that DHEA acted at the extracellular surface of the membrane. Moreover, DHEA did not act through protein phosphatases to inhibit the capsaicin-induced current. Furthermore, the stereoisomer of DHEA, 5-androsten-3α-ol-17-one (3α-DHEA), failed to inhibit the capsaicin-induced current, producing instead a potentiating effect on the capsaicin response, demonstrating that the interaction of steroids with the capsaicin receptor is stereospecific. The inhibitory action of DHEA on the capsaicin-induced current may provide a basis for reducing capsaicin receptor-mediated nociception.

Introduction

Capsaicin, the main pungent ingredient in hot chili peppers, activates a distinct subpopulation of primary sensory neurons, with somata in dorsal root, trigeminal as well as nodose ganglia (Szallasi and Blumberg, 1999). Capsaicin exerts its effects by binding to distinct cell-surface receptors. Electrophysiological studies from dorsal root ganglion neurons demonstrate that capsaicin generates an inward current with subsequent membrane depolarization and action potential generation (Heyman and Rand, 1985). This excitation is mediated by the opening of a nonselective cationic channel that is largely permeable to Na^+, K^+, and Ca^{2+} (Oh et al., 1996). In addition to capsaicin, the capsaicin receptor, transient receptor potential vanilloid subfamily 1 (TRPV1), also responds to other noxious stimuli including heat and extracellular protons (Tominaga et al., 1998). Capsaicin receptors have attracted particular attention by virtue of their proposed role in the transduction of painful stimuli (Szolcsanyi, 1990) and inflammatory thermal hyperalgesia (Caterina et al., 2000; Davis et al., 2000). A modulation of the capsaicin receptor present on the peripheral endings of nociceptors could therefore affect the detection and the transmission of pain messages by the somatosensory system as well as the formation of inflammation-induced heat hyperalgesia. Understanding the mechanisms underlying the modulation of the

capsaicin receptor by various agents may offer new therapies for treatment of pain.

Steroid hormones are known to influence profoundly the neuronal excitability. Although the effects of steroids have been thought to be mediated by genomic steroid response elements (McEwen, 1991), evidence has been accumulated to indicate that many steroids influence the neuronal excitability via direct effects on excitatory and inhibitory amino acid receptors (Majewska et al., 1986; Wu et al., 1990; Wu et al., 1991; Park-Chung et al., 1997). In particular, the neurosteroid dehydroepiandrosterone (5-androsten-3β-ol-17-one; DHEA) which has been claimed to be a "super hormone" modulates both N-methyl-D-aspartate (NMDA) receptor- and γ-aminobutyric acid type A ($GABA_A$) receptor-mediated responses in brain (Baulieu and Robel, 1996). Compared to the extensive studies of DHEA effects on amino acid receptors, very little is known about interaction of DHEA with the capsaicin receptor.

Using the whole-cell voltage-clamp technique and pharmacological methods, the specific aims of our study were (1) to determine whether DHEA modulates the capsaicin receptor-mediated current in acutely dissociated rat dorsal root ganglion (DRG) neurons; (2) to explore the mechanism of action of DHEA; (3) to examine the involvement of protein phosphatases in modulation by DHEA of the capsaicin response; (4) to determine whether DHEA acts intracellularly to modulate the capsaicin response;

and (5) to investigate structure-activity requirements for interactions of steroids with the capsaicin receptor.

Materials and Methods

Preparation of Rat Dorsal Root Ganglion Neurons. Animal care was consistent with the guidelines set by the Laboratory Animal Center of National Cheng Kung University. All experimental procedures were approved by the Animal Research Committee of Medical College of the National Cheng Kung University. Dorsal root ganglion neurons were acutely dissociated from Sprague-Dawley male rats (200-300 g). In some experiments, dorsal root ganglion neurons obtained from female rats of similar body weight were also used. The procedures used have been described previously (Wiley et al., 1993) with some minor modifications. Briefly, rats were anesthetized with urethane (1.2 g/kg body weight, intraperitoneally). Thoracic and lumbar dorsal root ganglia were rapidly removed, treated with 1 mg/ml Sigma type IA collagenase in Ca^{2+}/Mg^{2+} free Hank's balanced solution (GIBCO BRL, Grand Island, NY) supplemented with 4.2 mM $NaHCO_3$, 1 mM sodium pyruvate, 20 mM HEPES, and 3 mg/ml bovine serum albumin for 45 min at 37^{o}C, and pelleted by centrifugation (900 rpm, 4 min). The resulting pellet was suspended in Dulbecco's modified Eagle's medium (DMEM) (GIBCO BRL, Grand Island, NY) supplemented with 26.2 mM $NaHCO_3$, 1 mM sodium pyruvate , 20 mM HEPES and 5% fetal bovine serum (GIBCO BRL, Grand Island, NY), triturated gently to disperse the cells, and then centrifuged.

Cell pellet was resuspended in DMEM supplemented with 26.2 mM $NaHCO_3$, 1 mM sodium pyruvate , 20 mM HEPES, 100 units/ml penicillin, 100 μg/ml streptomycin, 1.8 mM $CaCl_2$, and 10% fetal bovine serum. Cell suspension (0.5 ml/3 ganglia) was plated on Corning 35-mm polystyrene tissue culture dishes and incubated at 37°C in an atmosphere of 5% CO_2/95% air. An additional 1-ml of medium was added after 30 min. Neurons were used in experiments within 1-10 hr after plating.

Electrophysiological recordings. Electrophysiological experiments were carried out at room temperature (23-25°C) in 35-mm tissue culture dishes on the stage of an inverted phase-contrast microscope. Whole-cell currents were recorded by the whole-cell variant of the patch clamp technique. Electrode resistance was 2-4 MΩ when filled with the intracellular solution containing (in mM): 140 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 10 HEPES, and 4 MgATP (pH adjusted to 7.2 with KOH). Sucrose was added to make osmolarity 320 mosM. In experiments such as determination of concentration-response curves for capsaicin in the presence and absence of 50 μM DHEA (Fig. 2) in which high concentrations of capsaicin were used, the intracellular solution was replaced with a Cs^+-containing pipette solution (in mM, 140 CsCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 10 HEPES, and 4 MgATP, pH adjusted to 7.2 with CsOH and osmolarity to 320 mosM with sucrose). The bath solution contained (in mM): 150 NaCl,

5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 D-glucose (pH adjusted to 7.4 with NaOH and osmolarity to 340 mosM with sucrose). All solutions passed through 0.2 μm Millipore filters before use.

Recordings were made using an Axopatch-1D patch clamp amplifier (Axon Instruments, Foster City, CA). Cells with series resistance greater than 10 MΩ were rejected. Only cells with resting membrane potential more negative than –40 mV and input resistance in excess of 150 MΩ were used. Unless otherwise specified, all recordings were made with the cell membrane potential clamped at –50 mV. Currents were filtered at 1 kHz using an 8-pole Bessel filter and digitized (4 ms/point) using an on-line data acquisition system (pClamp 6.0, Axon Instruments, Foster City, CA).

Drugs and Drug application. Drug solutions were applied to single neurons by pressure ejection (15 psi) from 7-barrel pipets (Chan and Farb, 1985). All steroids were purchased from Steraloids (Wilton, NH), with the exception of progesterone (Sigma, St. Louis, MO). Capsaicin, capsazepine, okadaic acid, and cyclosporin A were purchased from Tocris Cookson (Bristol, UK). Cyclophilin A was purchased from Sigma (St. Louis, MO). In the experiments such as determination of whether DHEA acts intracellularly and whether DHEA inhibits the capsaicin response via protein phosphatase (PP) activation, a saturating concentration (200 μM) of DHEA and the

selective PP inhibitors including okadaic acid (1 μM), cyclosporin A (50 nM) and cyclophilin A (20 nM) were, respectively, added in the pipette solution to allow them to readily reach their intracellular sites of action. Stock solutions of capsaicin, capsazepine, cyclosporin A, and steroids were prepared in dimethyl sulfoxide (final concentration, 0.5%, v/v). To obviate the possible effect of dimethyl sulfoxide on the capsaicin-induced current, all the other drug solutions, including external buffer (in the pressure pipettes), also contained 0.5% dimethyl sulfoxide. In all experiments, neurons received a 10-s pre-pulse of either external buffer or steroid solution, followed by a 30-s application of either capsaicin or capsaicin plus steroid, and further by a 20-s pulse of external buffer solution. A period of 4 min was allowed between successive applications of capsaicin.

Data Analysis. The degree of modulation of the capsaicin-induced current by steroid, the percent change, was defined as $(I'/I - 1)$ X 100%, where I and I' were, respectively, the capsaicin-induced current in the absence and presence of steroid. I was the average of control responses obtained before and after the capsaicin response in the presence of steroid. In most cases, complete or nearly complete reversal of the steroid effect was obtained after washout. DHEA concentration-response curve was determined by plotting the percentage of inhibition of the capsaicin-induced current as a function of

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the DHEA concentration. Data were fitted with the Hill equation: (% inhibition)/(% inhibition)$_{max}$ = [DHEA]^{n_H}/([DHEA]^{n_H} + $EC_{50}^{n_H}$), where [DHEA] is the concentration of DHEA, n_H is the Hill coefficient, and EC_{50} is the concentration by which the half-maximal inhibition is produced. Similarly, capsaicin concentration-response curves in the presence and absence of 50 μM DHEA were constructed by plotting the normalized capsaicin-induced current as a function of the capsaicin concentration. Each set of data were fitted with the Hill equation: I/I_{max} = [capsaicin]^{n_H}/([capsaicin]^{n_H} + $EC_{50}^{n_H}$), where I_{max} is the maximal normalized current, [capsaicin] is the concentration of capsaicin, n_H is the Hill coefficient, and EC_{50} is the concentration by which the half-maximal normalized current is induced. Throughout, results were expressed as the mean ± SEM. Data were compared statistically by Student's *t* test. *P* values less than 0.05 ($p < 0.05$) were considered as indicative of significance.

Results

DHEA Inhibits the Capsaicin Receptor-Mediated Current. At a holding potential of –50 mV, capsaicin activated inward currents (> 100 pA) in 68% (268/392) of neurons with diameters of 26-43 μm, which reversed at or near 0 mV in standard recording solutions, as expected for Na^+, K^+ and Ca^{2+}-mediated currents (data not shown). The currents induced by 100 nM capsaicin were completely blocked by 10 μM capsazepine (n = 4), consistent with activation of capsaicin receptors. In agreement with the capsaicin binding site being located at the intracellular surface of the receptor (Jung et al., 1999), the current induced by 100 nM capsaicin reached slowly to its full amplitude. The effect of DHEA on the current induced by 100 nM capsaicin is illustrated in Fig. 1. Pressure application of 100 μM DHEA rapidly and reversibly inhibited the capsaicin response (Fig. 1A). DHEA alone produced little or no direct response. To determine whether the effect of DHEA is sex-dependent, we examined and compared the effects of 100 μM DHEA on the 100 nM capsaicin-induced current in male and female rat neurons. Results revealed that the magnitude of reduction of the capsaicin response (93 ± 0.7%, n = 6) by DHEA in female rat neurons was similar to that (94 ± 1.3%, n = 6) observed in male rat neurons.

To quantitatively evaluate the potency and efficacy of DHEA for capsaicin

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receptors, pooled data were used to construct the concentration-response curve for inhibition of the 100 nM capsaicin response by DHEA. DHEA inhibited the capsaicin-induced current in a concentration-dependent manner, with an EC_{50} of 6.7 μM, a maximal inhibition of 100%, and a Hill coefficient of 0.83 (Fig. 1B). The threshold concentration for an effect of DHEA on the capsaicin-induced current was 0.1-1 μM. The maximal effect on the capsaicin response was achieved at approximately 100 μM.

Effect of DHEA on the Capsaicin Concentration-Response Curve. To investigate whether inhibition of the capsaicin response by DHEA is competitive, pooled data were used to construct concentration-response curves for capsaicin in the presence and absence of 50 μM DHEA. In this experiment, the cell was held at –30 mV to reduce the amplitude of the capsaicin-induced current and KCl was replaced with CsCl in the pipet solution to minimize the drift of the baseline current due to voltage-dependent K^+ fluxes at this depolarizing potential. In addition, all responses were normalized to the peak current induced by 0.5 μM capsaicin to obviate cell-to-cell variability with respect to the maximal current induced by capsaicin. As illustrated in Fig. 2, DHEA increased the capsaicin EC_{50} (2.12 μM with DHEA versus 0.70 μM without DHEA) with little effect on the normalized capsaicin maximal response (2.82 with DHEA versus 2.81 without DHEA).

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Effect of DHEA on the Capsaicin Response in the Presence of Intracellular Protein Phosphatase Inhibitors. Dephosphorylation of the capsaicin receptor by protein phosphatases (PPs) has been demonstrated to reduce the capsaicin response in rat sensory neurons (Docherty et al., 1996; Koplas et al., 1997). To examine whether DHEA inhibits the capsaicin response via PP activation, we first assayed the effect of DHEA (100 μM) on the capsaicin (100 nM) response in the presence of intracellular okadaic acid (1 μM), a selective PP1/2A inhibitor. We found that addition of okadaic acid in the pipette solution stabilized the capsaicin-induced current (data not shown) and did not significantly influence the inhibitory effect of DHEA on the capsaicin-induced current (Fig. 3A). Fifteen min after cell dialysis, the average inhibition produced by extracellular DHEA with okadaic acid inside was 94 ± 1.0% (n = 8), which did not significantly differ from that (94 ± 1.3%, n = 6) measured without intracellular okadaic acid ($p > 0.05$, unpaired *t*-test) (Fig. 3B). Similarly, inclusion of the selective PP2B inhibitor cyclosporin A (50 nM) plus cyclophilin A (20 nM) in the electrode buffer stabilized the capsaicin-induced current (data not shown) and did not significantly affect inhibition by extracellularly applied DHEA (100 μM) of the capsaicin (100 nM)-induced current (91 ± 2.1%, n = 7 with inhibitor versus 94 ± 1.3%, n = 6 without inhibitor).

The Site of Action of DHEA. To determine whether DHEA acts intracellularly to inhibit the capsaicin response, we tested the effect of a saturating concentration (200 μM) of intracellular DHEA on inhibition of the capsaicin response by extracellularly applied DHEA (100 μM). In this experiment, 1 μM okadaic acid was added in the pipette solution to prevent possible tachyphylaxis of the capsaicin-induced current. Under this condition, inclusion of DHEA in the electrode buffer had no significant effect on the capsaicin-induced current at 16 min after the cell was ruptured and did not block the effect of extracellular DHEA (Fig. 4A). In six cells, average currents induced by 100 nM capsaicin at 4, 8, and 16 min after cell rupture were, respectively 1554 ± 416.5, 1524 ± 336.0, and 1595 ± 352.0 pA, which did not differ significantly from that (1607 ± 451.3 pA) immediately (0 min) after cell rupture ($p > 0.05$, paired *t*-test). In addition, the average inhibition produced by extracellular DHEA with DHEA inside was 93 ± 2.9% (n = 6), which was not significantly different from that (94 ± 1.0%, n = 8) measured without intracellular DHEA ($p > 0.05$, unpaired *t*-test) (Fig. 4B).

Structure-Activity Relationships for Negative and Positive Modulation by Steroids. We also examined the effects of other chemically related steroids, including progesterone and some metabolites of DHEA (Table 1). Not all steroids inhibited the capsaicin response. Progesterone (100 μM) did not exert any significant effect on the

capsaicin response (Fig. 5A and Table 1). 5α-Androstan-3β-ol-17-one and 5β-androstan-3β-ol-17-one, reduced derivatives of DHEA, had activity similar to that of DHEA, whereas DHEA sulfate (DHEAS) and pregnenolone sulfate (PS) produced lesser effect. Thus, a double bond at C-5 is not required, and stereochemistry at C-5 is not critical for inhibition, whereas addition of a sulfate group at C-3β results in weaker activity. Interestingly, the stereoisomer of DHEA, 5-androsten-3α-ol-17-one (3α-DHEA), failed to inhibit the capsaicin-induced current, producing instead a potentiating effect on the capsaicin response (Fig. 5B and Table 1). In contrast to testosterone (one of DHEA metabolites), which produced substantially less inhibition than did DHEA, the female sex steroid hormone 17β-estradiol markedly potentiated the capsaicin response.

Discussion

The present study demonstrates that the neurosteroid DHEA concentration-dependently inhibits the capsaicin-induced current in acutely dissociated rat DRG neurons. In addition to the capsaicin receptor, the $GABA_A$ receptor is also negatively modulated by DHEA (Demirgoren et al., 1991; Baulieu and Robel, 1996; Kroboth et al., 1999). Inhibition by DHEA of the GABA-induced current is concentration-dependent, with an EC_{50} of 35 μM and a maximal inhibition of 68% (Demirgoren et al., 1991), indicating that DHEA is less potent and efficacious at the $GABA_A$ receptor than at the capsaicin receptor (EC_{50} = 6.7 μM; maximal inhibition = 100%; Fig. 1B). Like DHEA, its sulfated derivative, DHEAS, also antagonizes the GABA response, but with greater potency and efficacy (Demirgoren et al., 1991). This contrasts with our observation that DHEAS has lesser activity at the capsaicin receptor than DHEA (Table 1). These results, together with the findings that progesterone potentiates the GABA response (Wu, et al., 1990) but has little effect on the capsaicin response (Fig. 5A and Table 1), suggest that structure-activity requirements for steroid interaction with the capsaicin receptor and the $GABA_A$ receptor are different.

The high lipophilicity of the steroids raises the possibility that the effect of DHEA is due to the nonspecific mechanism of action, such as perturbation of the

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membrane lipids surrounding the capsaicin receptor protein. However, we have shown that progesterone does not produce any significant effect on the capsaicin response (Fig. 5A and Table 1). This finding indicates that inhibition of the capsaicin-induced current by DHEA is a specific effect. Moreover, in contrast to the inhibitory effect of DHEA on the capsaicin response, the stereoisomer of DHEA, 3α-DHEA, potentiates the capsaicin-induced current (Fig. 5B and Table 1), arguing that the interaction of steroids with the capsaicin receptor is stereospecific. This high degree of structural and stereochemical specificity for steroid effects suggests a specific site of interaction closely associated with the capsaicin receptor. The mechanism underlying potentiation by 3α-DHEA of the capsaicin response and the site of its action remain to be studied. Potentiation of the capsaicin response by 3α-DHEA could be due to an increase in the capsaicin maximal response, to a decrease in the capsaicin EC_{50} or to a combination of both effects. 3α-DHEA could act through the DHEA binding site or a distinct site to potentiate the capsaicin response. We are continuing to evaluate each possibility.

Because the whole-cell recording suffers from the washout effect in which intracellular second messengers critical to channel modulation are lost during the diffusional exchange between the cytoplasm and pipette solution, our observation that DHEA rapidly and reversibly inhibits the capsaicin response when neurons are recorded

in the whole-cell configuration, together with the fact that application of DHEA alone never induces any detectable membrane current, suggests that the inhibitory effect of DHEA on the capsaicin response is probably not mediated by intracellular steroid receptors or second messenger systems and that a specific membrane DHEA receptor such as the G protein-coupled receptor recently identified in bovine aortic endothelial cells (Liu and Dillon, 2002) is probably not involved.

Clinical studies have demonstrated sex differences in pain responses (Feine et al., 1991; Cepeda and Carr, 2003). Most recently, Frot et al. (2004) reported that women experienced capsaicin-induced pain as more intense than men did. The mechanisms underlying these sex differences are not clearly understood. One possibility is that sex differences in pain perception are mediated by sex differences in sensitivity of nociceptive neurons to steroid hormones such as DHEA. However, our observation that DHEA exerts similar inhibitory effect on the capsaicin response in male and female rat DRG neurons suggests that the effect of DHEA is sex-independent and excludes the possibility that differential modulation by DHEA of the capsaicin receptor in male and female neurons is the cause of sex differences in pain perception. An alternative explanation is that sex differences in pain responses may result from differential modulation of the capsaicin receptor by different sex steroids. Indeed, this idea is

confirmed by our data that the male sex steroid testosterone inhibits slightly, but the female sex steroid 17β-estradiol potentiates dramatically the capsaicin response in rat DRG neurons (Table 1). Since 17β-estradiol is the metabolite of testosterone, the opposite effects of these two steroids on the capsaicin response indicates that aromatization converts an inhibitory steroid to an excitatory steroid, suggesting that aromatase could play an important role in regulating the capsaicin receptor activity in the mammalian nervous system.

There are several potential sites at which DHEA could exert its blocking action including: (1) competitive inhibition at the capsaicin binding site and (2) non-competitive inhibition or allosteric modulation at a distinct site. First, DHEA increases the capsaicin EC_{50} with little effect on the capsaicin maximal response (Fig. 2), demonstrating that the depressive action of DHEA is competitive. Second, intracellular DHEA affects neither the capsaicin response nor inhibition by extracellularly applied DHEA of the capsaicin response (Fig. 4), indicating that the DHEA modulatory site is most likely to be on the external surface of the membrane. This contrasts with the binding site for capsaicin being present in the intracellular surface of the receptor (Jung et al., 1999; Jordt and Julius, 2002; Jung et al., 2002) and suggests that DHEA allosterically inhibits the capsaicin response via a distinct site. However, recent studies

on HEK293T cells expressing rat capsaicin receptor TRPV1 have shown that the recombinant capsaicin receptor can be activated by extracellularly applied capsaicin even in the presence of a saturating concentration of intracellular capsaicin (Vyklický et al., 2003), indicating that, in addition to intracellularly located capsaicin binding sites, there exists at least one extracelluar site, which needs to be occupied to activate the receptor. Therefore, it is also possible that DHEA may act through this extracellular site to competitively inhibit the capsaicin receptor-mediated response. In addition to DHEA, ruthenium red (Dray et al., 1990; Amann and Maggi, 1991), capsazepine (Bevan et al., 1992), and the neurosteroid PS (Table 1) also negatively modulate the capsaicin receptor. Inhibition of the capsaicin response by ruthenium red (Bevan et al., 1992) and PS (paper submitted for publication) is non-competitive, indicating that DHEA and these two compounds do not act through a common site. Like DHEA, antagonism of the capsaicin response by capsazepine is competitive (Bevan et al., 1992). Whether DHEA acts through the capsazepine modulatory site to inhibit the capsaicin response needs further investigation.

Considerable evidence suggests that pro-inflammatory prostaglandins (PGs), such as PGE_2, sensitize capsaicin responses in rat sensory neurons through an activation of cAMP-dependent protein kinase (PKA) to phosphorylate the capsaicin receptor

(Pitchford and Levine, 1991; Lopshire and Nicol, 1998; Bhave et al., 2002). In contrast, dephosphorylation of the capsaicin receptor either by PP2B or by ATP-free pipette solutions reduces the capsaicin response in rat dorsal root ganglion neurons (Docherty et al., 1996; Koplas et al., 1997; Piper et al., 1999). Therefore, it is possible that DHEA inhibition of the capsaicin-induced current might be mediated by PP activation to dephosphorylate the capsaicin receptor. However, inclusion of PP1/2A (Fig. 3) or PP2B inhibitors in the pipette buffer do not significantly affect inhibition of the capsaicin response by DHEA, demonstrating that PP1/2A and PP2B are not involved in the inhibitory action of DHEA.

Free DHEA and DHEAS are metabolically interconvertible by sulfotransferase for conjugation and sulfatase for hydrolysis in many tissues (Baulieu, 1996; Kroboth et al., 1999). DHEA and DHEAS, which are collectively designated DHEA(S), are secreted in high amounts in humans and a few other primates. The highest reported plasma concentration of DHEA(S) in humans is about 10 μM (Parker and Odell, 1980; Guillemette et al., 1996; Hornsby, 1997), which is close to our EC_{50} value (6.7 μM) for DHEA effect on the capsaicin response (Fig. 1B). In sharp contrast, plasma concentrations of DHEA(S) in rats are < 0.1 μM (Robel and Baulieu, 1995; Guillemette et al., 1996), below the concentration range at which neuromodulation of the capsaicin

receptor is observed. However, because neurosteroids such as DHEA(S) can be synthesized locally in the nervous system of rats from cholesterol (Robel and Baulieu, 1995; Kroboth et al., 1999), high local DHEA(S) concentrations could occur. Moreover, there is evidence that neurosteroid levels can vary in response to environmental changes. For example, increases in brain DHEA(S) levels have been observed after exposure of male rats to females (Robel and Baulieu, 1995) and after stress (Corpechot et al., 1981). Therefore, it is likely that DHEA under physiological conditions may modulate the capsaicin receptor in the mammalian nervous system.

In summary, our results show, for the first time, that the neurosteroid DHEA acts competitively and extracellularly to inhibit the capsaicin receptor-mediated current in rat DRG neurons. This indicates the possibility of an endogenous modulation by DHEA of capsaicin receptor-mediated signaling in the nervous system where capsaicin receptors are located. Given the prominent role for the capsaicin receptor in nociception (Szolcsanyi, 1990) and inflammation-induced heat hyperalgesia (Caterina et al., 2000; Davis et al., 2000), DHEA may reduce the capsaicin receptor-mediated pain sensation and contribute to prevent the formation of inflammatory thermal hyperalgesia.

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Footnotes:

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Figure Legends

Fig. 1. DHEA inhibits the capsaicin response. A, DHEA (100 μM) inhibits rapidly and reversibly the current induced by 100 nM capsaicin (Cap). Horizontal bar above each trace, period of drug application. B, concentration-response curve for inhibition of the capsaicin (100 nM) response by DHEA. Data points, percentage change in peak current in the presence of DHEA (mean of four to seven experiments). Error bars, standard errors. Error bars are not indicated when smaller than the size of the circle. DHEA concentration-response curve is fitted with the Hill equation (see Materials and Methods). Curve-fit analysis reveals an EC_{50} of 6.7 μM, a maximal inhibition of 100%, and n_H of 0.83.

Fig. 2. Antagonism of the capsaicin response by DHEA is competitive. Concentration-response curves for capsaicin in the presence and absence of 50 μM DHEA. All responses are normalized to the peak current (*) induced by 0.5 μM capsaicin. Data points, normalized peak currents (mean of four to eight experiments). Error bars, standard errors. Error bars are not indicated when smaller than the size of the circle. Each set of data points is fitted with the Hill equation (see Materials and Methods). In the absence of DHEA, EC_{50} = 0.70 μM, I_{max} = 2.81, and n_H = 1.78. In the

presence of DHEA, EC_{50} = 2.12 μM, I_{max} = 2.82, and n_H = 1.30.

Fig. 3. DHEA inhibition of the capsaicin response is not mediated by activation of PP1/2A. A, inclusion of the selective PP1/2A inhibitor okadaic acid (1 μM) in the electrode buffer does not significantly affect inhibition by extracellularly applied DHEA (100 μM) of the capsaicin (100 nM)-induced current. B, average data for the effect of extracellularly applied DHEA (100 μM) on the capsaicin-induced current in the absence (Control) and presence (OA inside) of intracellular okadaic acid (1 μM). Number of cells is indicated in parentheses.

Fig. 4. DHEA acts extracellularly to inhibit the capsaicin response. A, dialyzing the inside of the cell with a saturating concentration (200 μM) of DHEA influences neither the capsaicin (100 nM)-induced current nor inhibition by extracellularly applied DHEA (100 μM) of the capsaicin-induced current. Time after cell rupture is shown above each tracing. B, average data for the effect of extracellularly applied DHEA (100 μM) on the capsaicin-induced current in the absence (Control) and presence (DHEA inside) of intracellular DHEA (200 μM). Number of cells is indicated in parentheses.

Fig. 5. Effects of progesterone and 3α-DHEA on the capsaicin response. **A,** application of 100 μM progesterone (P) has no significant effect on the 100 nM capsaicin-induced current. B, 3α-DHEA (50 μM) enhances rapidly and reversibly the current induced by 100 nM capsaicin.

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TABLE 1

Effects of steroids on the 100 nM capsaicin-induced current

Holding potential is –50 mV. Values are means ± standard errors. Number of cells is indicated in parentheses.

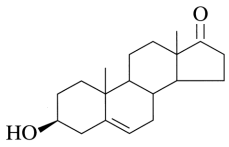
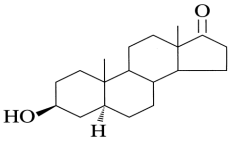
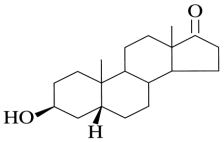
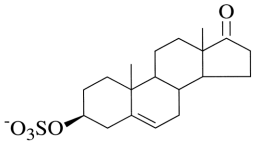
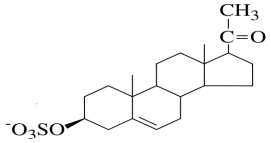
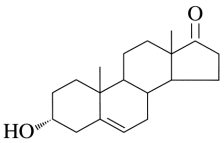
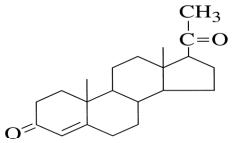
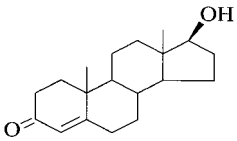
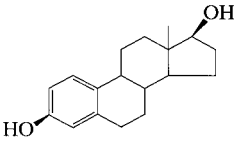
Steroid	Structure	Change of response (%)
DHEA (50 μM) (100 μM)		-73 ± 6.3 (6) -94 ± 1.3 (6)
5α-Androstan-3β-ol-17-one (100 μM)		-92 ± 1.2 (4)
5β-Androstan-3β-ol-17-one (100 μM)		-94 ± 1.5 (5)
DHEAS (100 μM)		-45 ± 6.4 (7)
PS (100 μM)		-57 ± 5.3 (14)
3α-DHEA (50 μM)		+78 ± 5.7 (5)
Progesterone (100 μM)		+1 ± 6.7 (7)
Testosterone (100 μM)		-25 ± 4.2 (7)
17β-Estradiol (100 μM)		+174 ± 31.8 (8)

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Fig. 1

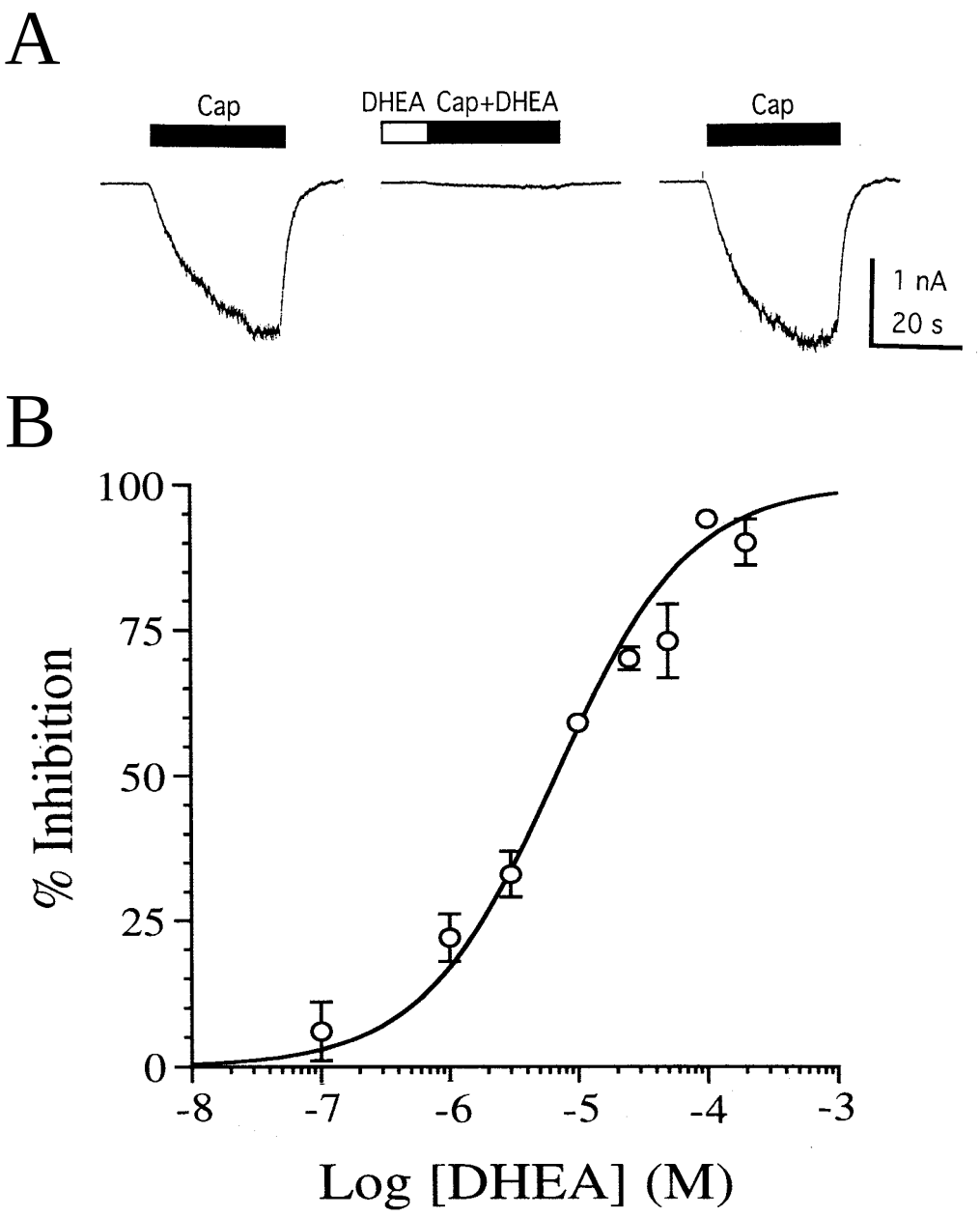

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Fig. 2

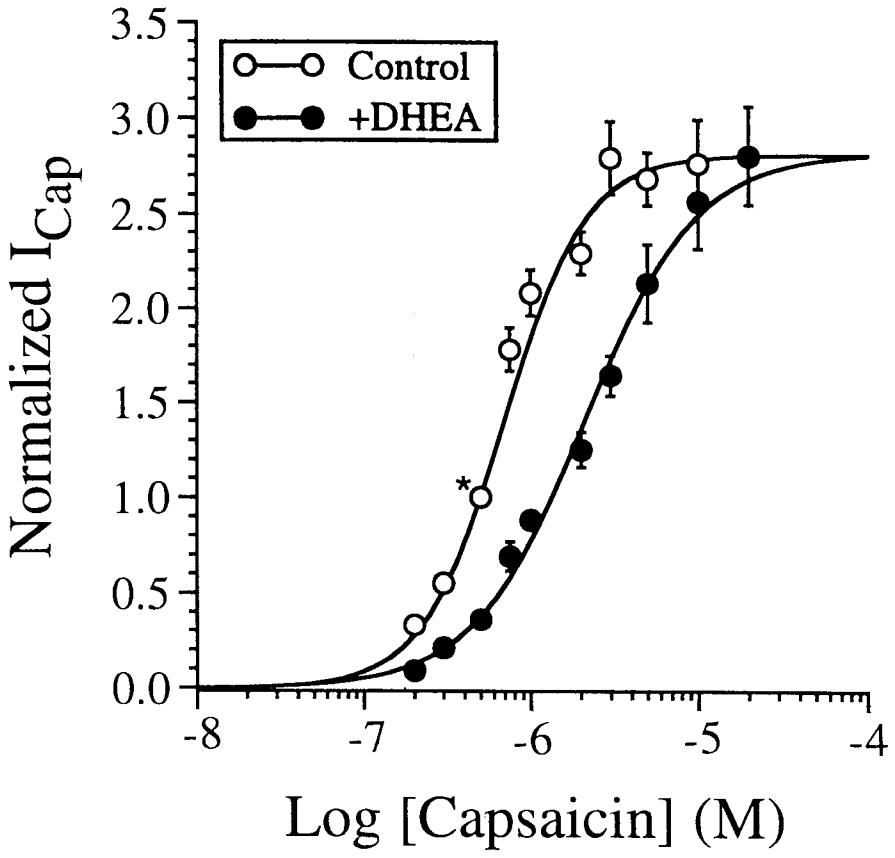

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Fig. 3

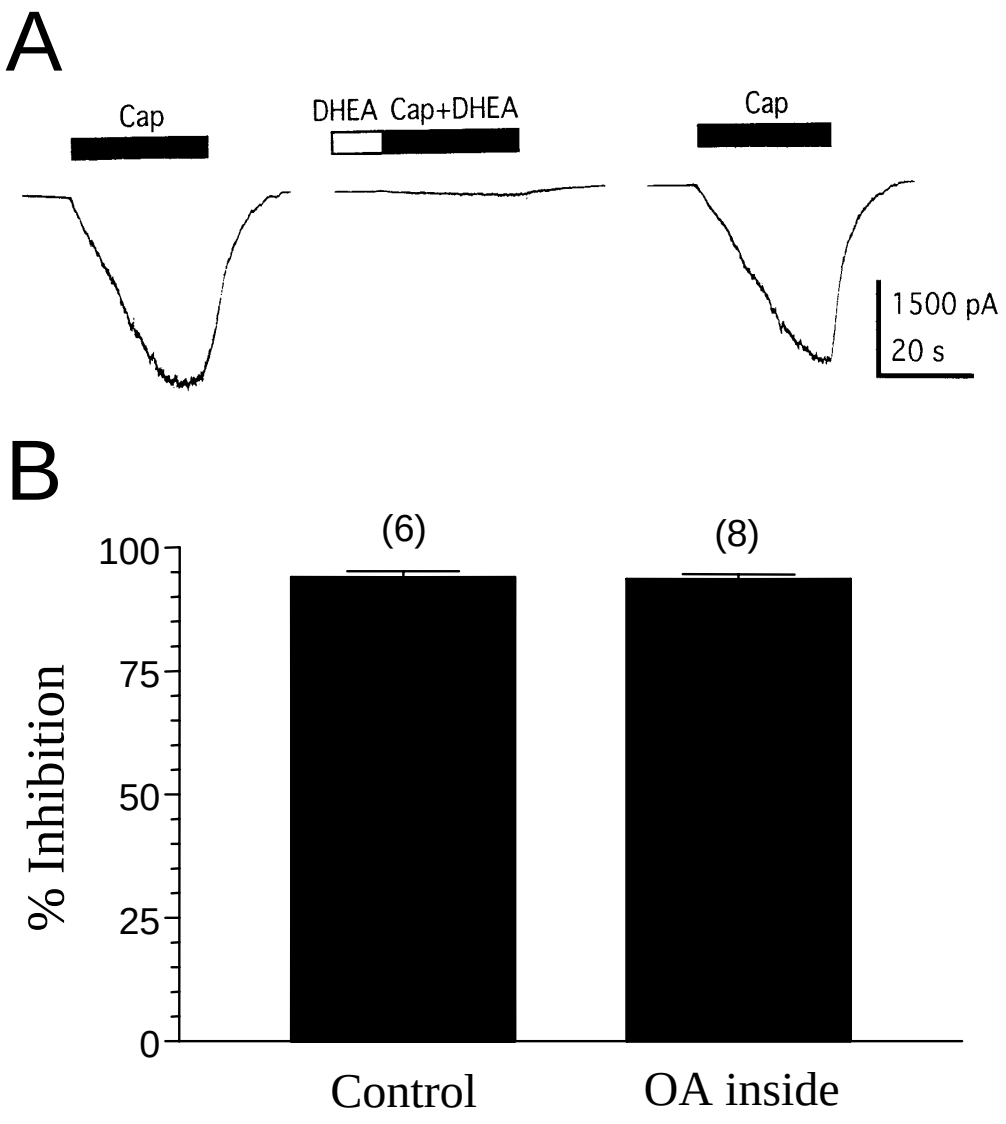

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Fig. 4

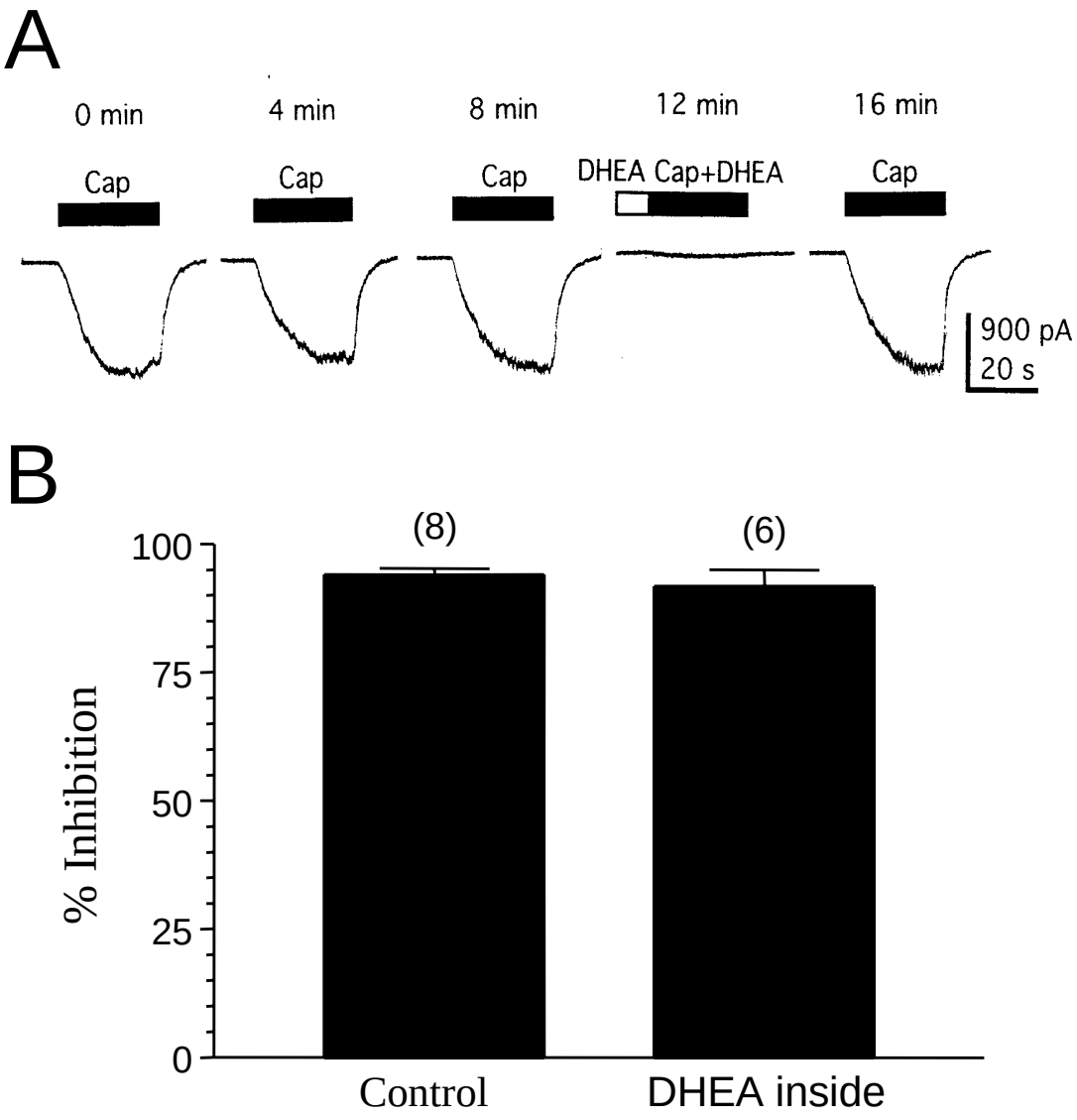

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Fig. 5