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Effects of estrogen on calcium signaling in molecular pathways of pain; Importance of TRPV1 cation channels

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Abstract

Pain is an unpleasant and uncomfortable sensation that informs us that something is wrong and overrides other neuronal signals. Nociceptive pain is an unconscious response of sensory receptors to noxious stimuli. Transient Receptor Potential (TRP) channels are a family of non-selective cation channels located at nociceptive sensory nerve endings that respond to thermal, mechanical, or chemical stimuli with threatening/irritating potential. Depending on the type and intensity of the stimulus, different TRP proteins alter calcium ion permeability and modulate cellular response patterns. TRP Vanilloid Receptor-1 (TRPV1), which has the most crucial role in the activation of pain transmission pathways in this family, is sensitive to vanilloid-like chemicals, temperature (>43^{0}C), and low pH.

Recent studies indicate that gonadal hormones are actively involved not only in reproductive behavior and sex determination but also in all physiological systems. Current literature shows that gonadal hormones play active roles not only in reproductive behavior and sex determination but also in all physiological systems. Estrogen, the most potent of these hormones, has been the subject of numerous studies due to its vital effects on the nervous system, such as neuronal viability, excitability, and perception of somatosensory stimuli. Although the literature shows that estrogen has essential roles in the modulation of pain, there is insufficient evidence regarding the molecular pathways of its effect. This review aims to investigate the importance of calcium signaling in the molecular pathways of pain and the effects of estrogen on TRPV1 cation channels that alter the calcium ion permeability of nociceptors.

Keywords: TRPV1 channels; Estrogen; Calcium; Pain; Nociceptors

Estrogen as a Neuroendocrine Hormone

Neuroendocrine Effects of Estrogen

Estrogens are a family of steroid hormones synthesized mainly from cholesterol. Estrogen is a female sex hormone that in the non-pregnant female is secreted in large amounts from the ovaries and small amounts from the adrenal cortex, whereas, in pregnancy, it is secreted in large amounts from the placenta [1]. There are three types of estrogen; 17β-estradiol (E2) is released from the ovaries in the adult female. Estrone (E1), released from the ovaries. It is also formed in small amounts by peripheral deposition of androstenedione released from the adrenal cortex. Estriyol (E3) is formed by metabolizing E2 and E1 in the liver. E2 is the most critical estrogen type. It has estrogenic effects 12 times stronger than E1 and 80 times stronger than E3 [1-3]. Androstenedione is converted to E1 and partly to testosterone in the ovaries. Testosterone is converted to 5α-dihydrotestosterone by 5α-reductase or E2 by aromatase [4]. In addition to ovaries, many extragonadal tissues such as fat, bone, liver, kidney, kidney, lung, brain, and blood vessels also produce estrogen [5].

Many studies have shown that estrogen is a highly effective hormone in physiological systems, especially female secondary sex characteristics, cardiovascular system, skeletal muscle system, and immune system [6-10]. Estrogen is synthesized at a lower level in males than females but plays essential roles in basic cellular mechanisms in both sexes [11]. Estrogen has important roles in the central and peripheral nervous system. Receptors for this hormone are widely distributed in the amygdala, hippocampus, cortex, cerebellum, spinal cord, and peripheral nerves [12]. Estrogen regulates neuromodulatory and neurotransmitter substances' synthesis, expression, and receptor activity [13]. It is also involved in the development of neurons and the regulation of synaptic functions [14]. When estrogen level decreases, synaptic transmission decreases, and structural changes occur in neurons. Another critical role of estrogen in the nervous system is to regulate glucose utilization by controlling total cerebral and cerebellar blood flow rate through receptors in endothelial cells in brain vascular structures [15].

Studies have shown that estrogen is indispensable for hippocampal neurogenesis [16, 17]. It was determined that the number of dendritic spins in hippocampal CA1 pyramidal cells increased by 30% in rats administered estrogen for two days. Thus, the number of excitatory synaptic connections increased [17]. In a study with mice lacking the E2 β receptor, it was determined that there were deficiencies in the amount and organization of neurons in the cerebral cortex of these mice, with obvious morphological abnormalities [18]. In addition, they have significant regulatory effects on most neurotransmitter systems [19]. It has been determined that E2 is also essential in sensory conduction, and it has been reported that E2 treatment significantly increased sensory conduction in the pudendal nerve in ovariectomized rats [20].

Estrogen can exert its effects through genomic and non-genomic mechanisms [12]. In genomic mechanisms, the hormone binding to its receptor inside the cell produces long-term effects by providing specific gene transcription and protein synthesis. In contrast, estrogen changes the conductance of ion channels or neuronal excitability in non-genomic mechanisms by connecting with the primary messenger [21]. The intracellular receptors of estrogen are ER-α and ER-β. In addition, the G-protein-coupled receptor (GPR30) on the cell membrane mediates the rapid non-genomic effects of estrogen [21]. For example, it can cause a receptor to dissociate from its effector system (estrogen prevents the effects of μ-opioid and γ- aminobutyric acid B [GABA B] receptors by dissociating them from their effector systems) [22] or can activate the intracellular second messenger system by binding some membrane-associated receptors. It is accepted that estrogen regulates pain via second messenger systems such as cAMP/protein kinase A, protein kinase C, mitogen-interacting protein kinase (MAPK), and extracellular signal regulatory kinase (erk) [7].

Estrogen and Modulation of Pain

Pain is a highly complex sensation that can occur due to many causes and involves many physiological mechanisms. The International Association for the Study of Pain (IASP) defines pain as ‘an unpleasant, uncomfortable sensory and emotional experience associated with or described in the context of actual or potential tissue injury.’ Nociception is defined as ‘the unconscious response of sensory receptors to a noxious stimulus’ [2]. Pain usually starts with activating sensory receptors in somatic or visceral structures called nociceptors, which transmit nociceptive (pain) information to the CNS [23]. Reducing the activity of nociceptors or blocking their input to the CNS can alleviate these inputs' complex sensory and emotional consequences.

In recent years, interest in the effects of estrogen on the nervous system has increased, and studies on its functions in pain pathways have intensified. It has been recognized that estrogen receptors are widely present in the nervous system and that this hormone plays a vital role in the autonomic and sensory systems [12, 24]. Estrogen has been reported to modulate sensory inputs at the primary afferent level [25]. The distribution of the two types of estrogen receptors (ER-α and

ER-β) in the body differs. Both of these receptors have an affinity for E2, and their effects may be opposite to each other [12]. The effects of estrogen are observed depending on which receptor is prevalent in the tissue. If the expression of these receptors co-occurs in a tissue, ER-β can suppress the expression of ER-α and inhibit its effects [26, 27]. Immunohistochemical studies have determined that ER-α is more abundant than ER-β in nerve cells and ganglia and that this receptor mediates more estrogen's effects on the nervous system [11]. Therefore, it is thought that estrogen modulates pain via the α receptor. Studies with ER-α-deficient or ER-β-deficient mice have determined that estrogen can cause pro-nociceptive effects via ER-α and anti-nociceptive effects via ER-β. In addition to these, considering the rapid effects of estrogen via GPR30, it may be normal to find different results in the literature regarding the effects of this hormone on pain.

Human and animal studies have shown that the pain threshold is lower in females than in males, and the intensity and duration of pain response is higher in females than in males; in addition, the frequency of chronic pain syndromes is higher in females than in males [7]. In studies using various irritating stimuli such as heat, chemical irritants, pressure, formalin, and Capsaicin (CAP), pain threshold and pain tolerance were lower in females than males in healthy subjects [28]. Furthermore, epidemiological studies have shown that women have visceral pain with greater severity and frequency than men. Moreover, the frequency of chronic pain conditions such as migraine, temporomandibular disease, fibromyalgia, and arthritis is higher in females [29]. This gender difference in pain conditions is likely to be due to the effects of gonadal steroid hormones [29-31]. In addition, the finding that pain threshold and pain tolerance were lower in women than men with various irritant stimuli such as heat, chemical irritants, pressure, formalin, and CAP suggests that TRPV1 channels activated by these irritants mediate the pain modulation of E2.

The results of the studies on the modulation of nociception caused by uncomfortable stimuli by estrogen show that E2 is effective in pain regulation. In contrast, the results of a few studies are uncertain [7]. In experimentally estrogen-withdrawn rats, it has been shown that estrogen deficiency decreases the pain threshold to the disturbing stimulus and increases the response rate and intensity [32-36]. At the same time, a few studies have found no difference between the groups [37, 38]. CAP effects in rat dorsal root ganglion neurons (DRG) were mildly inhibited by the male sex hormone testosterone but significantly potentiated by the female sex hormone E2 [39], revealing that CAP intradermal injection dose-dependently induced pain sensitization in males and females and that pain onset was 3-4 times higher in males than in females, using comparable, appropriate doses [40]. These experimental findings show sex differences in pain threshold and pain tolerance to various irritant stimuli such as CAP, strengthening the evidence that TRPV1 channels are one of the molecular pathways of pain modulation by E2.

E2 -pain-opioid receptor relationship is another topic that researchers have focussed on in relation to pain [41]. Endogenous ligands of opioid receptor agonists such as morphine and exogenous agonists of these receptors are the most effective substances in visceral pain and have antinociceptive and analgesic effects. There is ample evidence that sex differences play an important role in nociception and opioid-induced antinociception [29]. The effective half-dose of morphine was found to be higher in female rats than male rats in visceral pain [42, 43] and resistant somatic pain [43-45]. In a few studies, it was determined that sex differences did not alter the analgesic effects of morphine [46]. The gender dimorphic effects of morphine on pain modulation suggest that the gonadal steroid E2 contributes to pain sensitization [28]. It has been reported that strong pain-relieving opioids such as morphine increase prolactin secretion in the hypothalamus-pituitary-gonadal axis and decrease gonadal hormone levels by decreasing LH secretion [47].

The relationship between E2 and serotonin is another subject that has been extensively studied [48, 49]. It is suggested that estrogen increases the production of serotonin and decreases its degradation and reuptake. In the absence of progesterone, estrogen withdrawal may trigger migraine, while it is also possible that progesterone modulates gamma-aminobutyric acid (GABA) activity, pain, and pain perception [50]. Gonadal hormones and pain modulation have been a topic of interest to countless researchers. Due to the complexity of pain, its multi-causal and multi-focal nature, and the existence of different types of pain, pain-related studies have also been multifaceted. There have been many studies on the effects of gender differences on nociceptive sensitivity, and different results have been obtained from both human and animal experiments. The literature reports that estrogen has essential roles in pain modulation and provides evidence that this modulation may be both pro-nociceptive and anti-nociceptive (**Table 1**).

TRP Channels

Nociceptive sensory nerves have a large number of receptors at their endings. These receptors respond to mechanical, chemical, or thermal stimuli with a threatening/irritating potential. Most of these are members of

Table 1.

Pain-regulating Effects of Estrogen		References
Estrogen regulates the synthesis of pain-related transcription factors	➢ Modulation of the expression and activity of TRP channels by gonadal hormones is essential in the gender dimorphism observed in migraine. ➢ Estrogen regulates the synthesis of pain-related receptors and proteins. ➢ Nociceptive stimuli regulate the synthesis of gonadal hormone receptors.	[51] [52 - 55] [56]
Decreased nociception due to estrogen effect	➢ In rats, E2 treatment reduced pain. ➢ Ovariectomy induced thermal and mechanical hyperalgesia, while E2 supplementation reduced pain. ➢ Visceral pain activity and trigeminal neuron sensitivity increased during the low estrogen period. ➢ Pain sensitivity was found to be higher in women during the low estrogen period.	[22, 57] [33, 58-61] [62] [63]
Increased nociception due to estrogen effect	➢ CAP-induced acute nociceptive pain sensitization was found to be higher in female rats than in male rats. ➢ Thermal and mechanical nociceptive sensitization is higher in female rats. ➢ In experimental visceral pain, the pain threshold is significantly lower in females than males. ➢ Women have been found to have higher pain sensitivity and lower tolerance to pain than men.	[40] [64, 65] [28, 66] [67]

a family of non-selective cation channels called Transient Receptor Potential (TRP) channels [2]. Today, TRP cation channels, which contain approximately 30 members in mammals, are defined as a new cation channel family. TRP cation channels play a role in many vital processes such as regulation of blood pressure and smooth muscle tone, renal Ca^{2+} / Mg^{2+} transmission, perception of pungent taste and odorous compounds, mechanical changes, pain, heat, taste, odor, sound and light [68-71].

According to the amino acid similarities of channel proteins, TRP family is divided into 7 subfamilies [72]; TRP ankyrin (TRPA) of one member, TRP canonical (TRPC) consists of seven subfamilies, TRP melastatin (TRPM) of eight subfamilies, TRP mucolipin (TRPML) of three subfamilies, TRP polycystin (TRPP) of three subfamilies and TRP vanilloid (TRPV) of six subfamilies [73, 74]. TRP cation channels have been shown to play an essential role in the physiopathology of many diseases. Although there are many structural similarities between these channels, the activators of the subtypes of these channels are very different. The basic structure of TRP channels, except for some TRPPs, is proposed to be formed by the regions crossing the membrane 6 times. The hydrophobic ring between the TM5 and 6 segments is thought to be the ion channel-forming pore, and the COOH and NH2 ends are located in the cytoplasm. TRPs form homo- or heterotetrameric structures to function [69, 75]. In vivo, the assembly of functional TRP channel complexes is governed by homo/hetero multimerization and complex formation with structural proteins [76]. These formations explain the differences between physiological functions proposed in different tissues and the functional properties of TRP channels observed in heterologous expression systems.

The TRPV family has six members in mammals. They are divided into four subgroups: TRPV1/TRPV2, TRPV3, TRPV4, and TRPV5/6 [77, 78]. TRPV family members function as tetrameric complexes. There are 3-5 NH2-terminal ankyrin repeats [79] in all TRPV channels. TRPV2 is a stretch-activated channel that acts as a mechanosensor in vascular smooth muscle [80] and is likely involved in skeletal and cardiac muscle degeneration and pain pathways [81]. TRPV4 is involved in mechanotransduction, thermoregulation, osmoregulation, and basal Ca^{2+} homeostasis [82, 83]. The

most Ca^{2+}-selective channels of the TRP family are the TRPV5 and TRPV6 channels, which are under Ca^{2+} regulation [84]. In the absence of extracellular calcium, these two channels conduct monovalent cations, whereas, under physiological conditions, they conduct calcium. TRPV5 is essential for Ca^{2+} reabsorption in the kidney, while TRPV6 is vital in the intestine [85]. TRPV1 has attracted the most attention in relation to pain modulation among these channels.

TRPV1 is an ion channel receptor that can produce CAP and CAP-like effects and is activated by high temperature (>43^0C) and acid (pH≤5). TRPV1 is commonly found in sensory neurons and ganglia. It has also been shown to be present in other neurons and various non-neuron cells [86, 87]. The influx of Ca^{2+} ions into the cell is stimulated when these receptors are activated by various stimuli [88, 89]. There are two types of vanilloids: endogenous and exogenous [90]. Examples of exogenous vanilloids include CAP, the active ingredient of red hot pepper, resiniferatoxin in cactus, and anandamide, whereas endogenous vanilloids include inflammation-induced pH changes and temperature changes [91, 92].

Estrogen, Calcium Signaling, and TRPV1

TRPV1 cation channels are essential regulators of nociceptive and inflammatory pain, and it is well-known that TRPV1 expression, activation, and modulation are associated with pain [93]. Many physical and chemical stimuli, such as high temperature, extracellular and intracellular pH imbalances, and CAP, which is a direct stimulus of the channel, can activate the channels, and a nociceptive response occurs. TRPV1 has been found to play an essential role in detecting and regulating noxious stimuli in DRGs and neurons, especially in nociceptive afferent fibers [94]. In mice lacking the TRPV1 gene, it has been shown that acute pain to an irritating stimulus is regulated through these channels [95]. Studies with TRPV1 antagonists have shown that this cation channel is effective in various pain models and plays a vital role in pain transport and modulation [96]. TRPV1 can be activated not only by disturbing external stimuli but also by internal stimuli such as low pH, high temperature (>43^0C), and anandamides [97], and inflammatory agents such as prostaglandins and bradykinin also activate these channels [98]. In TRPV1 gene-deficient mice, thermal nociception and inflammation-induced thermal hyperalgesia were found to be low, while mechanical allodynia due to inflammation and nerve damage were not different [90]. Honore et al. (2005) showed that allodynia due to neuropathy and inflammation was also reduced in rats [99].

Experiments show that female animals are more sensitive to capsaicin-induced pain than male animals [32]. E2 is thought to be involved in the difference between the two sexes in this type of pain. A study in rat DRG neurons shows that E2 mediates the development of sensitization of female rats to capsaicin-induced acute pain and that E2 potentiates this sensitization through the capsaicin receptor [40]. Another study showed that capsaicin injected into the tail of male and female rats produced a dose-dependent thermal hyperalgesia in both male and female rats, with higher capsaicin sensitization in females than males [100]. Prolonged exposure to estradiol in neuronal cultures from DRGs of female rats inhibits activation of the TRPV1 ion channel, which is central to nociceptive transmission in the DRG, and cultured trigeminal ganglion cells from ovariectomized rats exhibit increased sensitivity to capsaicin [101]. This increases the importance of calcium signaling in the molecular pathways of pain and the importance of investigating the effects of estrogen on TRPV1 cation channels that alter the calcium ion permeability of nociceptors.

Calcium is defined as the general carrier of information that directs all cellular processes from cell formation to cell death [102]. Today, the role of calcium has been demonstrated in cellular processes such as cell division, cellular motility, hormone secretion, metabolism, nervous system development and neurotransmission, protein turnover, gene expression, and programmed cell death [103, 104]. A minor disruption in calcium homeostasis leads to impaired functioning of critical cellular pathways and many diseases [105]. While Ca^{2+} acts as a second messenger generated in active cells, it can also act as a true first messenger outside the cell membrane, such as a hormone or growth factor. The function of calcium for signal generation is directly proportional to the increase in its cytosolic amount [104]. The Ca^{2+} concentration in the cell can be increased or decreased by simultaneously opening or closing several oppositely directed ion channels. The increase in intracellular calcium concentration is mediated by ion channels on the surface of the endoplasmic reticulum (ER) and sarcoplasmic reticulum (SR), in addition to ion channels located in the cell membrane. Conversely, a decrease in Ca^{2+} concentration is mediated by several exchange mechanisms (Na^+/ Ca^{2+}), resulting in a density difference or energy expenditure through Ca^{2+} ATPases located in the cell membrane and ER/SR [106]. Calcium, which plays a regulatory role in almost all cellular processes, is vital in many steps of steroid synthesis, including estrogen [107].

TRP channels determine the total amount of calcium by regulating the entry/exit of calcium into the cell under the

influence of the endocrine system and internal and external environmental factors. Under unfavorable conditions such as extreme cold/heat, low pH, various plant active substances, and animal poisons, these channels are activated and cause calcium ions to pass into the cell. Changes in the amount of calcium ions cause the initiation of physiological or pathological processes in the cell, including steroid hormone synthesis and secretion [108]. TRP channels are involved in the control of steroid hormone synthesis, and these hormones also affect the activity of TRP channels [109]. This interaction between steroid hormones and TRP channels leads to important events such as developmental anomalies, body temperature regulation, and sex differences that alter sensitivity to sensory stimuli [108]. In a study with ovariectomized and non-ovariectomized rats treated with E2, it was determined that allodynia was significantly reduced after intrahippocampal injection of TRPV1 antagonists capsazepine and 5-iodoresiniferatoxin [61]. The significant increase in hippocampal TRPV1 expression in temporomandibular disorders suggests that this channel may regulate central pain processing and that E2 is also the cause of higher pain sensitivity in females [61].

Conclusions

In recent years, studies on the functions of estrogen in pain pathways have intensified. Estrogen has been reported to be important in pain regulation through the modulation of analgesic systems such as serotonin and opioids. Studies on the role of estrogen in pain modulation have provided evidence that estrogen may be pro-nociceptive or anti-nociceptive, but the effects are not clear. There have been many studies on the effects of gender differences on nociceptive sensitivity, and different results have been obtained from both human and animal experiments. Therefore, it is essential to determine the possible molecular mechanisms of estrogen in pain modulation. TRP ion channels, for which there is increasing evidence of their multifaceted functional importance, are widely expressed in the nervous system, mainly in sensory systems, and have been implicated in many physiological and pathophysiological events. In this context, TRP channels are likely to be one of the molecular mechanisms of estrogen in pain modulation. Therefore, understanding the role of TRP channels in estrogen modulation of pain may help in the development of novel therapeutic strategies for pain. Recent studies have highlighted the potential of TRP channels, particularly TRPV1, as therapeutic targets for pain.

In conclusion, modulation of TRP channels has emerged as a new area of research with important implications for developing effective treatments for analgesia. Further studies are needed to unravel the complex mechanisms underlying the relationship between TRP channels and estrogen modulation of pain and to identify specific TRP channel subtypes as potential therapeutic targets.

Conflict of Interest Statement

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Author Contributions

All of the authors declare that they have all participated in the design, and execution of the paper and that they have approved the final version.

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