17β-Estradiol Rapidly Enhances Bradykinin Signaling in Primary Sensory Neurons In Vitro and In Vivo


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ABSTRACT

Many studies have demonstrated that premenopausal women are at increased risk for various pain disorders. Pain-sensing neurons, termed “nociceptors,” in the trigeminal ganglia (TG) and dorsal root ganglia (DRG) express receptors for inflammatory mediators and nocuous physical stimuli and transmit signals for central processing of pain sensation. Estrogen receptors (ERs) are also expressed on nociceptors in the TG and DRG, and there is ample literature to suggest that activation of ERs can influence pain mechanisms. However, the mechanism for ER modulation of nociceptor activity is incompletely understood. The aim of this study was to characterize the effect of 17β-estradiol (17β-E2) on signaling of the inflammatory mediator bradykinin (BK) in primary cultures of rat sensory neurons and a behavioral model of thermal allodynia in rats. Here, we show that exposure to 17β-E2 rapidly (within 15 min) enhanced responses to BK in vitro and in vivo. The 17β-E2-mediated enhancement of BK signaling was not blocked by the transcription inhibitor anisomycin and was mediated by a membrane-associated ER. The effect of 17β-E2 to enhance BK responses required activation of β1-containing, RGD-binding integrins. These data show that 17β-E2 rapidly enhances inflammatory mediator responses both in vitro and in vivo and suggest that 17β-E2 acting at primary sensory pain neurons may participate in regulating the sensitivity of women to painful stimuli.

Introduction

Epidemiological studies have demonstrated that women are at increased risk for a variety of pain disorders, including temporomandibular joint disorder, fibromyalgia, headache, and arthritis, among others (Fillingim et al., 2009). Under controlled experimental conditions, women are generally more sensitive to pain-causing stimuli than men (Cairns and Gazerani, 2009; Fillingim et al., 2009), and this sex difference extends to many studies in animals as well (Wiesenfeld-Hallin, 2005; Cairns and Gazerani, 2009). The mechanisms for sex differences in pain responsiveness are numerous, complex, and far from understood. Although some of these differences may be accounted for by cognitive and sociocultural gender differences, there is strong evidence for significant biological differences in pain perception and processing between the sexes. It is noteworthy that many studies have identified the important role played by sex hormones, in particular estrogen (Wiesenfeld-Hallin, 2005; Craft, 2007; Cairns and Gazerani, 2009; Fillingim et al., 2009).

The actions of estrogen on nociception are also complex and multifactorial. Both pronociceptive and antinociceptive effects have been attributed to estrogen in human and animal models (Wiesenfeld-Hallin, 2005; Craft, 2007; Cairns and Gazerani, 2009; Fillingim et al., 2009). Such apparently contradicting differences in the effects of estrogen on nociception may be related to differences among pain conditions (inflammatory, neuropathic, etc.), differential actions of estrogen at multiple levels of the pain transmission/perception pathways, and time-dependent effects of estrogen, including genomic versus nongenomic (rapid) signaling.

Of the many places within the pain transmission pathway that estrogen may act, there is abundant evidence indicating that estrogen can regulate the activity of the primary sensory neurons involved in pain transmission, termed nociceptors. Estrogen receptors (ERs) are expressed in nociceptors of the trigeminal ganglia (TG) and dorsal root ganglia (DRG) (Yang...
et al., 1998; Bereiter et al., 2005), and treatment with 17β-
estriadiol (17β-E2) influences a variety of functions and cel-
lar processes in nociceptors such as expression of trkA
mRNA (Liu et al., 1999), expression of calcitonin gene-
related peptide mRNA and protein (Gangula et al., 2000),
extracellular signal-regulated kinase activity (Liverman et
al., 2009), calcium mobilization (Chaban and Micevych,
2005), and transient receptor potential cation channel V1
(TRPV1) function (Xu et al., 2008). Furthermore, local
17β-E2 injection into the temporomandibular joint reduces
nociceptive behavioral responses to intrajoint administration
of formalin (Fávaro-Moreira et al., 2009), suggesting
estrogen’s effect on nociceptors is functionally relevant.

Classic ERs are members of the nuclear receptor super-
family and are comprised of α (ERα) and β (ERβ) subtypes,
which, when activated by estrogen, act as transcription fac-
tors to regulate protein synthesis in target tissues. With
respect to pain, estrogen has been shown to regulate the
expression of a number of proteins involved in nociception
(Aloisi and Bonifazi, 2006). The effects mediated by this
“genomic” pathway typically have rather long latencies for
onset of action and extended duration (hours to days). How-
ever, recent evidence strongly suggests that estrogen can
have rapid (within seconds) nongenomic effects that seem to
be mediated by plasma membrane-associated ERs (Hammes
and Levin, 2007). In DRG neurons in vitro, estrogen, acting
via ERs associated with the plasma membrane, rapidly (with
5 min) reduces ATP-mediated intracellular calcium mobil-
ization (Chaban and Micevych, 2005). Estrogen also rapidly
activates extracellular signal-regulated kinase (Liverman et
al., 2009) and interferes with exchange protein activated by
cAMP-mediated activation of protein kinase Ce (Hucho et
al., 2006). These data suggest that estrogen can exert multiple
actions (genomic and nongenomic) on primary sensory neu-
trons to regulate pain neurotransmission.

The effects of estrogen on nociception may also differ de-
pending on the nature of the pain stimulus (Cairns and
Gazerani, 2009; Fillingim et al., 2009), and both pronocicep-
tive and antinociceptive effects have been reported for in-
fammatory pain (Straub, 2007). Pain induced by inflamma-
tion results from the release of a myriad of inflammatory
mediators, such as bradykinin (BK), prostaglandins, cyto-
kines, and proteases, and it is possible that the effect of
estrogen could differ depending on the specific inflammatory
mediator involved or biological outcome observed.

To begin to understand the contradictory effects of estro-
gen on inflammatory pain, we assessed the ability of 17β-
estriadiol (17β-E2) to regulate the effects of BK in primary
cultures of TG neurons in vitro and in a behavioral model of
thermal allodynia in vivo. We found that 17β-E2 rapidly
enhanced both BK signaling in TG neurons and BK-induced
thermal alloydiana, suggesting that rapid-onset actions of ex-
rogen, acting at primary sensory nerve terminals, may con-
tribute to enhanced pain sensitivity in females.

Materials and Methods

Materials. Fetal bovine serum was purchased from Gemini Bio-
products (Calabasas, CA). All other tissue culture reagents were
from Invitrogen (Carlsbad, CA). 17β-Estradiol-6-O(carboxymethyl)
oxime-BSA (17β-E2-BSA) was purchased from Sigma-Aldrich (St.
Louis, MO). For in vitro experiments, 17β-E2-BSA was diluted in 50
mM Tris-HCl (pH 7.2) to a working stock solution of 10 μM based on
BSA concentration. For in vivo experiments, 17β-E2-BSA was dis-
solved in saline and administered to the rat hindpaw at doses of 30
or 50 μg that correspond to approximately 100 or 150 ng of 17β-E2,
respectively, based on the molecular weight of BSA. All other drugs
and chemicals were purchased from Sigma-Aldrich.

Animals. The animal study protocol was approved by the Insti-
tutional Animal Care and Use Committee of the University of Texas
Health Science Center at San Antonio and conformed to Interna-
tional Association for the Study of Pain and federal guidelines. Adult
male and female Sprague-Dawley rats, 200 to 250 g, were purchased
from Charles River Laboratories, Inc. (Wilmington, MA). Ovariecto-
tomy was performed at Charles River Laboratories, Inc. Experi-
ments with ovariectomized (OVX) rats were performed at least 2
weeks after surgery. Animals were housed for 1 week with food and
water available ad libitum before experiments.

Rat Trigeminal Ganglion Culture. Primary cultures of rat TG
cells were prepared as described previously (Patwardhan et al., 2006;
Berg et al., 2007a,b). In brief, rats were sacrificed by decapitation,
and TGs were rapidly removed and chilled in Hank’s balanced salt
solution (HBSS; Ca2+, Mg2+ free) on ice. TGs were washed with
HBSS, digested with 3 mg/ml of collagenase for 30 min at 37°C, and
centrifuged. The pellet was further digested with 0.1% trypsin for
15 min at 37°C, pelleted by centrifugation (5000 g for 5 min), and
resuspended in Dulbecco’s modified Eagle’s medium (high glucose)
containing 100 ng/ml of nerve growth factor (Haran, Indianapolis, IN),
10% fetal bovine serum, 1% penicillin/streptomycin, 1% l-glutamine,
and the mitotic inhibitors uridine (7.5 μg/ml) and 5-Fuoro-2’-de-
oxyuridine (17.5 μg/ml). After trituration to disrupt tissue, the cell
 suspension was seeded on polylysine-coated 24- or 48-well plates.
Media were changed 24 and 48 h after plating and every 48 h
thereafter. Nerve growth factor and serum were removed 24 h before
experiments. Cells were used on the 5th or 6th day of culture.

Measurement of Inositol Phosphate Accumulation. BK-
stimulated inositol phosphate (IP) accumulation in TG cultures was
measured as described previously (Patwardhan et al., 2006; Berg et
al., 2007a). Cells grown in 24- or 48-well plates were labeled with
2μCi/ml [3H]myoinositol for 24 h before experiments. After labeling,
cells were rinsed three times with 1 ml of HBSS that contained 20
mM HEPES and 20 mM lithium chloride (LiCl) and were preincu-
bated in HBSS/LiCl at 37°C in room air for 15 min. BK (various
concentrations) was added to a final volume of 500 μl, and cells were
further incubated for 25 min. Where indicated, 17β-E2 was added
during the preincubation period. The incubation was terminated by
the addition of 200 μl of ice-cold formic acid, and total [3H]IPs
were separated with ion-exchange chromatography and measured
with liquid scintillation spectrometry. Data are expressed as accumula-
tion of total IPs (disintegrations per minute) or a percentage of basal
IP accumulation.

Behavioral Testing. Paw withdrawal latency (PWL) to a ther-
mal stimulus was measured with a plantar test apparatus (Har-
greaves et al., 1988) by observers blinded to the treatment allocation.
In brief, rats were placed in plastic boxes with a glass floor. After a
30-min habituation period, the plantar surface of the hindpaw was
exposed to a beam of radiant heat through the glass floor, and the
PWL was automatically determined by a photelectric cell. The rate
of increase in temperature of the glass floor was adjusted so that
baseline PWL values were close to 10 ± 2 s; cutoff time was 20 s.
Measurements were taken in duplicate at least 30 s apart, and the
average was used for statistical analysis. BK, Gly-Ary-Gly-Asp-Ser-
Pro (GRGDSP, integrin-blocking peptide), Gly-Ary-Gly-Asp-Ser-Pro
(GDGRSP, inactive, reverse-sequence integrin-blocking peptide),
and 17β-E2-BSA stock solutions were diluted in saline. 17β-E2
was diluted in peanut oil. All drugs were administered via intraplantar
injection at a final volume of 50 μl. To assess a role for gene tran-
scription in the mechanism of action of 17β-E2, we administered the
transcription inhibitor anisomycin (Sigma-Aldrich) via intraplantar
injection, 15 min before intraplan-
tar injection of 17β-estradiol. At a concentration of 10 μM, anisomycin inhibits protein synthesis by 99% (Grollman, 1967). Using a value of 1 ml as an estimate (likely an overestimate) of paw volume, we chose a dose of 5 μg/50 μl of anisomycin that would lead to a calculated concentration of anisomycin of 20 μM.

**Data Analysis.** For TG cell culture experiments, concentration-response data were fit to a logistic equation using nonlinear regression analysis to provide estimates of maximal response (R_max), potency (EC_50), and slope factor (n):

\[ R = \frac{R_{\text{max}}}{1 + (\text{EC}_{50}/[A])^n} \]

where R is the measured response at a given agonist concentration (A), R_max is the maximal response, EC_50 is the concentration of agonist that produces half-maximal response, and n is the slope index. Statistical differences in concentration-response curve parameters between groups were analyzed with Student’s paired t test. When only a single concentration was used, statistical significance was assessed by using one-way analysis of variance followed by Dunnett’s post hoc test (paired) using Prism software (GraphPad Software, Inc., San Diego, CA). p < 0.05 was considered statistically significant.

For behavioral experiments, time course data were analyzed with two-way analysis of variance, followed by Bonferroni’s post hoc test. Statistical inference was made when p < 0.05. Data are presented as mean ± S.E.M.

**Results**

**17β-E_2 Effects on BK Signaling in TG Cultures.** In TG cultures from OVX female rats, pretreatment with 17β-E_2 rapidly and significantly increased both the potency (∼8-fold) and the efficacy (∼2-fold) of BK to stimulate the phospholipase C (PLC)-IP pathway (Fig. 1). The pEC_50 values for BK were 8.00 ± 0.36 (10 nM) versus 8.87 ± 0.47 (1.3 nM) for the vehicle and 17β-E_2-pretreatment groups, respectively. The maximal response (E_max) to BK was increased from 48 ± 6% (vehicle pretreatment) to 87 ± 16% above basal by 17β-E_2 pretreatment. It is noteworthy that 17β-E_2 did not alter the potency or maximal response of BK in TG cultures derived from male rats (pEC_50: 9.64 ± 0.26 versus 8.89 ± 0.9; E_max: 86 ± 10% versus 86 ± 5% for vehicle- and 17β-E_2-pretreatment of male cultures, respectively). Thus, the effect of 17β-E_2 on TG cultures was clearly sexually dimorphic.

Figure 2 shows that a membrane-impermeable form of 17β-E_2 (17β-E_2 conjugated to BSA) also significantly enhanced the BK-PLC response. Pretreatment (15 min) with 17β-E_2-BSA enhanced the response to 100 nM BK by 2-fold, whereas pretreatment with 17β-E_2 increased the BK response by 3-fold.

**17β-E_2 Effects on BK-Induced Thermal Alloodynia.** Using observers blinded to treatment allocation, the intraplantar injection of BK in the hindpaw of OVX rats produced thermal allodynia at doses above 1 μg (Fig. 3). At effective doses, the thermal allodynia was rapidly induced but significantly enhanced by 17β-E_2 pretreatment. It is noteworthy that 17β-E_2-BSA enhanced the response to 100 nM BK by 3-fold. Pretreatment (15 min) with 17β-E_2-BSA enhanced the response to 100 nM BK by 2-fold, whereas pretreatment with 17β-E_2 increased the BK response by 3-fold.
Fig. 4. 17β-Estradiol rapidly enhances BK-induced thermal allodynia in OVX rats. A, time course of BK-induced thermal allodynia. OVX rats were injected intraplantarly with 17β-E2 (50 or 100 ng) or vehicle (peanut oil) 15 min before injection with a subthreshold dose of BK (1 μg). PWL was measured in duplicate every 5 min after each injection. Data are expressed as change from individual preinjection baselines and represent the mean ± S.E.M. of four to six animals per group. **, p < 0.01; ****, p < 0.001 versus vehicle. B, effect of estrogen on the dose-response curve for BK-induced thermal allodynia. OVX rats were injected intraplantarly with 17β-E2 (100 ng) or vehicle (peanut oil) 15 min before injection of BK (various doses, intraplantarly). PWL was measured in duplicate every 5 min after each injection. Data are expressed as change from individual preinjection baselines at the time of maximal BK effect (5 min post-BK) and represent the mean ± S.E.M. of four to six animals per group. Bulb intensity was adjusted for baseline PWL of 12.52 ± 0.22 s.

(within 5 min) and was transient, returning to baseline by 15 min after injection. The ED50 for BK was 2.8 μg.

Intraplantar injection of 17β-E2 to OVX rats at doses up to 100 ng did not produce a change in PWL over the measured time period (Fig. 4). However, 15-min pretreatment with 17β-E2 rapidly sensitized responses to BK, such that an inactive dose (1 μg) now produced a significant thermal allodynia with a magnitude similar to that observed with 10 μg of BK (Fig. 3 versus 4). Moreover, the BK-sensitizing effect of 17β-E2 was dose-related, with significant differences observed with pretreatment with either 50 or 100 ng of 17β-E2 into the ipsilateral hindpaw. A dose-response analysis indicated that 17β-E2 pretreatment increased the potency of BK by ~3-fold (ED50 2.8 μg versus 0.8 μg; Fig. 4B). Similar to the effect of 17β-E2, intraplantar injection of the membrane-impermeable 17β-E2-BSA (30 or 50 μg) did not produce a change in PWL but dose-dependently enhanced the response to BK (1 μg; Fig. 5). Injection of 17β-E2 into the contralateral hindpaw did not affect the BK response (Fig. 6), indicating that the action of 17β-E2 to enhance BK-induced thermal allodynia was peripherally mediated. The effect of 17β-E2 to enhance thermal allodynia in response to BK was not blocked by prior administration of the transcription inhibitor anisomycin into the ipsilateral hindpaw (Fig. 7).

RGD-Binding Integrins Mediate the Effect of 17β-E2 on BK Responses In Vitro and In Vivo. Integrins are expressed on many cell types, including afferent neurons, and the RGD subclass of these heterodimeric proteins regulates the signaling activity of several membrane-bound receptors, including opioid receptors on peptidergic TG neurons (Litvak et al., 2000; Short et al., 2000; Berg et al., 2007b). Accordingly, we evaluated whether disruption of the RGD subclass of integrins modulates the effect of 17β-E2 to enhance BK-stimulated IP accumulation in TG cultures derived from OVX rats (Fig. 8). Administration of an integrin antag-
Fig. 7. Pretreatment with the transcription inhibitor anisomycin has no effect on 17β-E₂-mediated enhancement of BK-stimulated thermal allodynia. OVX rats were pretreated (intraplantarly at time – 30 min) with anisomycin (5 μg) before pretreatment (intraplantarly at time – 15 min) with 17β-E₂ (100 ng) or vehicle (peanut oil). Fifteen minutes later (at time 0 min), animals were injected intraplantarly with a subthreshold dose of BK (1 μg in saline), and PWL responses were measured in duplicate every 5 min after each injection. Data are presented as change in PWL compared with individual preinjection paw baseline. Each point represents the mean ± S.E.M. of six animals per group. ***, p < 0.001. Bulb intensity was adjusted for baseline PWL of 12.44 ± 0.49 s.

Fig. 8. 17β-Estradiol enhancement of BK-PLC activity is blocked by integrin antagonists. A, effect of soluble RGD peptide. TG cultures from female rats were pretreated with Gly-Arg-Gly-Asp-Ser-Pro (soluble RGD peptide; 100 μg) before pretreatment (intraplantarly at time 15 min) with 17β-E₂ (50 nM) or vehicle for 15 min (37°C). Cultures were then exposed to BK (1 nM) for 25 min (37°C), and total IP accumulation was determined as under Materials and Methods. Data are the mean ± S.E.M. of three experiments. *, p < 0.05 compared with vehicle-vehicle condition; †, p < 0.05 compared with vehicle-17β-E₂.

Discussion

Although a role for estrogen in gender differences in pain sensitivity has been supported by numerous studies (Wiesenfeld-Hallin, 2005; Craft, 2007; Cairns and Gazerani, 2009; Fillingim et al., 2009), typically such estrogen effects have been attributed to the classic “genomic” pathway where an intracellular ER complex acts as a transcription factor to regulate protein synthesis. Acting via this classic pathway, the effects of estrogen generally are characterized as having a long latency for onset of action and a long duration of action, which persists even after estrogen is removed from the system. Here, we show that 17β-E₂ can rapidly (within minutes) increase BK signaling in primary cultures of sensory neurons in vitro and nociceptive responsiveness to BK in vivo. The rapid onset of action of 17β-E₂ suggests that the effect is not mediated by the classic “genomic” pathway. Over the past several years, an emerging body of evidence indicates that estrogen can also regulate cellular activity via ERs associated with the plasma membrane (Hammes and Levin, 2007). Typically, these effects are of short-onset latency (within seconds) and do not require changes in transcription. The effectiveness of a membrane-impermeable form of 17β-E₂ (17β-E₂ conjugated to BSA) to enhance BK signaling in TG cultures and nociceptive responses in vivo and the lack of effect of the transcription inhibitor anisomycin in vivo also support the hypothesis that the effect of estrogen is not mediated by the classic genomic pathway.

17β-E₂ administration to the rat hindpaw rapidly and significantly enhanced BK-induced thermal allodynia via a local mechanism of action restricted to the injected hindpaw. Given that ERs are expressed in primary sensory neurons (Yang et al., 1998; Bereiter et al., 2005) and together with the results from primary culture of sensory neurons, these data indicate that 17β-E₂ acts locally, likely at membrane-associated receptors on primary sensory neuron terminals, to rapidly enhance pain sensitivity to the inflammatory mediator BK.

Several studies have found that estrogen treatment can increase responsiveness to a variety of painful stimuli
Data are expressed as change in PWL compared with individual preinjection and represent the mean ± S.E.M. of 5 to 14 animals per group. *** p < 0.001 versus all other groups. Bulb intensity was adjusted for baseline PWL of 11.12 ± 0.27 s.

These actions seem to be mediated by classic estrogen receptors (ERα or ERβ or splice variants, thereof) that are associated with the plasma membrane or are present in the cytosol (Razandi et al., 1999; Clarke et al., 2000; Watson et al., 2002). Post-translational changes (e.g., palmitoylation) have been proposed to traffic ERα and ERβ subtypes to plasma membrane microdomains enriched in cholesterol, receptors, and signaling molecules, such as caveolae (Accionia et al., 2003; Razandi et al., 2003). Several signal transduction pathways have been implicated in rapid ER signaling, including mitogen-activated protein kinase, adenylyl cyclase, PLC, nitric oxide, and phosphatidylinositol 3-kinase (Kelly and Levin, 2001; for reviews, see Hall et al., 2001; Levin, 2002). Experiments to identify the signaling pathways that mediate estrogen's action on BK responsiveness are underway.

Here, we report that certain members of the RGD-binding family of integrins mediate the ability of 17β-E₂ to enhance BK signaling. Integrins are a heterogeneous class of cell-surface proteins expressed by virtually every cell type and are best known to be involved in the regulation of several vital cell functions, including adhesion, migration, proliferation, and differentiation (van der Flier and Sonnenberg, 2001). Recent evidence indicates that integrins can also regulate signaling by seven-transmembrane spanning receptors (Litvak et al., 2000; Short et al., 2000; Berg et al., 2007b).

Eighteen α and eight β subunits have been identified, forming at least 24 different αβ integrins (van der Flier and Sonnenberg, 2001). Integrins composed of α₁, α₅, α₆, α₁₀, or α₈ subunits (10 of the known 24 integrins contain one of these α subunits) bind to molecules containing an RGD sequence (van der Flier and Sonnenberg, 2001), which is exposed in extracellular matrix molecules such as fibronectin and vitronectin. Upon binding to an RGD sequence immobilized within extracellular matrix molecules, integrins become activated and signal to a variety of intracellular signaling cascades, including ion channels, kinases, and associated proteins (Coppolino and Dedhar, 2000; Juliano, 2002; Martin et al., 2002). However, soluble RGD-containing peptides or soluble antibodies against integrin subunits block integrin signaling (Chavis and Westbrook, 2001). Here, we found that soluble RGD peptides completely blocked the enhancement of BK signaling in vitro and in vivo without affecting BK signaling on its own. In addition, a soluble antibody directed against the β1 integrin subunit blocked the effect of 17β-E₂. These data suggest that 17β-E₂ regulation of BK signaling is mediated by RGD-binding integrins that contain the β1 subunit. It is noteworthy that application of a soluble antibody against the β5 integrin subunit enhanced BK signaling in both the absence and presence of 17β-E₂, and the effect on BK signaling was not further increased by 17β-E₂. These data suggest that β5-containing integrins provide tonic inhibition of BK signaling that can be overcome by activation of β1-containing integrins (by 17β-E₂) and underscores the complexity of integrin regulation of cellular functions.

The mechanism by which RGD-binding integrins participate in the regulation of BK signaling by 17β-E₂ is unknown. Integrins regulate a variety of intracellular protein kinase signaling cascades (Coppolino and Dedhar, 2000; Juliano, 2002; Martin et al., 2002) that, via cross-talk mechanisms, may regulate signaling or trafficking of the BK receptor system. Alternatively, integrins promote powerful changes in the organization of the cytoskeleton and can regulate the...
constituents of caveolin-containing membrane microdomains (Salandueva et al., 2007), which spatially restrict the distribution of, and signaling by, a variety of receptor systems (Allen et al., 2007). Receptor-mediated signaling can be either enhanced or decreased by trafficking of receptors and signaling molecule partners (e.g., G proteins) between membrane compartments (Allen et al., 2007). Consequently, by regulating the signaling molecule composition of membrane microdomains, integrins could regulate BK signaling. Although the present studies do not distinguish among these mechanisms, they provide strong documentation for a rapid estrogen-mediated regulation of BK signaling and function that critically involves mediation by the RGD class of integrins.

In summary, 17β-E₂ rapidly enhanced BK signaling in cultures of rat primary sensory neurons and enhanced BK-induced thermal allodynia in rats. The action of 17β-E₂ was probably nongenomic, mediated by cell membrane-associated ERs located on peripheral primary sensory nerve terminals, and required β1-containing integrins. Collectively, these results indicate that 17β-E₂ acting on primary sensory neurons may participate in the enhanced sensitivity of women to painful stimuli.

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References


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