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17β-estradiol attenuates α, β-meATP-induced currents in rat dorsal root ganglion neurons

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Abstract

The effects of 17β-estradiol on the α,β-me ATP-induced currents were studied on dorsal root ganglion (DRG) neurons using whole-cell recording technique. Three types of currents (transient, sustained or biphasic) were evoked by α,β-me ATP in acutely dissociated DRG neurons. When neurons were pre-incubated with 17β-estradiol (10–1000 nM) for 4 min, an inhibition of the transient current and the transient component of the biphasic current was observed. In contrast, 17β-estradiol did not have any significant effect on the sustained current evoked by α,β-meATP. The inhibitory effects were concentration-dependent, reversible and could be blocked by the estradiol receptor inhibitor, ICI 182,780 (1 μM). However, bovine serum albumin-conjugated 17β-estradiol (17β-estradiol-BSA, 10nM) failed to mimic the effects of 17β-estradiol. 17α-estradiol, the inactive isoform, did not have significant effects on αβ-meATP-induced currents, either. Sustained currents induced by ATP (100 μM) in nodose ganglion (NG), superior cervical ganglion (SCG) and otic ganglion (OTG) neurons were not affected by 17β-estradiol. These results suggest that the female gonadal hormone, 17β-estradiol, might participate in control of pain by modulating $P2X_3$ receptor-mediated events in sensory neurons.

Keywords: Estradiol; αβ-meATP; Dorsal root ganglion neurons

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Introduction

A number of chronic pain conditions such as irritable bowel syndrome, interstitial cystitis and orofacial pain are more prevalent in females than in males and the severity of pain fluctuates with the menstrual cycle, suggesting that gonadal hormones may affect the perception of painful stimulations. Sex-related differences in nociceptive responses have also been reported in rodents with females having stronger nociceptive reactions than males (Aloisi et al., 2001; Kim et al., 2000; Cairns et al., 2001). However, conflicting results have been reported regarding the role of gonadal steroid hormones. Several studies indicate a negative correlation between endogenous estrogen levels and pain threshold, with pain sensitivity increasing when estrogen levels are highest. For example, the threshold to evoke a visceromotor reflex or pressor response to colorectal distention (CRD) or to activate uterine and vaginal primary afferents is lowest in proestrus (Robbins et al., 1992; Sapsed-Byrne et al., 1996; Holdcroft et al., 2000). On the other hand, elevated estrogen levels reportedly increase thresholds to cutaneous stimuli (Martínez-Gómez et al., 1994) and decrease the percentage of escape responses to vaginal canal stimulation (Bradshaw et al., 1999) or ureteral calculosis (Giamberardino et al., 1997). Furthermore, elevated estrogen and progesterone, as occurring during pregnancy, is antinociceptive (Gintzler and Bohan, 1990; Dawson-Basoa and Gintzler, 1998). The reason for this contradictory relationship between gonadal hormones and pain sensitivity is unclear although the relative concentrations of individual gonadal hormones may play a role.

Estrogen receptors, which consist of two subtypes (ie, ER_α and ER_β), are widely distributed throughout the central and peripheral nervous systems (Bettini et al., 1992; Papka et al., 1997). It has been demonstrated that estradiol can modulate the gene expression of endogenous opioid peptides (Priest et al., 1995; Holland et al., 1998) and opioid neurotransmission (Eckersell et al., 1998). In addition to the genomic mode of effects, non-genomic mode of actions has also been reported. For example, estrogen has been shown to modulate rapid changes of ion-channels and G-protein-coupled neurotransmitter receptors (Eckersell et al., 1998; Kelly et al., 1998; Mermelstein et al., 1996; Sinchak and Micevych, 2001). The binding site of estrogen has also been reported in the plasma membrane and these membrane-associated receptors have similar pharmacology with those in nucleus (Pappas et al., 1995; Razandi et al., 1999).

It has been demonstrated that the peripheral sensory neurons express both estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β) (Taleghany et al., 1999), with ER-α being selectively localized in small-diameter neurons. Therefore, estrogen can potentially alter the nociceptive process at the primary afferent level.

ATP is implicated in peripheral sensory transduction of noxious stimuli by activating ATP-gated ion channels, namely P2X receptors (Dunn et al., 2001; Burnstock, 2000). Three types of responses to ATP have been observed in primary afferent neurons: transient, sustained and biphasic (having both transient and sustained components) (Dunn et al., 2001). Recently, Chaban et al. (2003) demonstrated that estradiol attenuated ATP-induced Ca^{2+} influx and this effect might be mediated through activation of membrane-bound ER-α receptors (Chaban et al., 2003). In this report, we have investigated the possible effects of estradiol on P2X receptor-mediated whole-cell currents in primary afferent neurons. Our observations suggest that estradiol selectively modulates homomeric $P2X_3$ receptor-meidated

transient currents without affecting homomeric $P2X_2$ or heteromeric $P2X_{2/3}$ receptor-mediated responses.

Materials and methods

Cell culture

4-6-week-old male rats were killed by inhalation of a rising concentration of CO_2. Lumbar dorsal root ganglia (DRG) were rapidly dissected out. In some experiments, superior cervical ganglia (SCG), nodose ganglia and otic ganglia (OTG) (Suzuki and Hardebo, 1991) were collected as well. The ganglia were desheathed, cut and incubated in 4 ml Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution with 10 mM Hepes buffer (pH 7·4) (HBSS; Life Technologies) containing 1·5 mg ml^{-1} collagenase (Class II, Worthington Biochemical Corporation, UK) and 6 mg ml^{-1} bovine serum albumin (Sigma, Poole, UK) at 37°C for 45 min. The tissue was then incubated in 4 ml HBSS containing 1 mg ml^{-1} trypsin (Sigma) at 37°C for 15 min. The solution was replaced with 1 ml growth medium comprising of L-15 medium supplemented with 10 % bovine serum, 50 ng ml^{-1} nerve growth factor, 0·2 % $NaHCO_3$, 5·5 mg ml^{-1} glucose, 200 i.u. ml^{-1} penicillin and 200 i.u. ml^{-1} streptomycin. The ganglia were then dissociated into single neurons by gentle trituration. The neurons were plated onto 35 mm Petri dishes coated with 10 μg ml^{-1} laminin (Sigma) and maintained at 37°C in a humidified atmosphere containing 5 % CO_2, and used within 30 hours.

Whole-cell voltage-clamp recording

Whole-cell currents were recorded at room temperature using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA) with membrane potential being held at −60 mV. External solution contained (in mM): NaCl 154, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, Hepes 10 and glucose 5.6 with pH adjusted to 7.4 using NaOH. Recording electrodes (resistance 2–4 MΩ) were filled with internal solution which contained (in mM): citric acid 56, $MgCl_2$ 3, CsCl 10, NaCl 10, HEPES 40, EGTA 1, tetraethylammonium chloride 10, and the pH was adjusted to 7.2 using CsOH (total Cs^+ concentration 170mM). Current signals were acquired using pClamp software (Version 6.1. Axon Instruments) and were plotted using Origin7 (Microcal, Northampton, MA, USA).

Drug application

Drugs were applied rapidly through a manifold comprising 7 capillaries made of fused silica coated with polyimide, with 250μm internal diameter (SGE, Milton Keynes, UK), connected to a single outlet made of the same tubing, which was placed about 200 μm from the cell. Solutions were delivered by gravity flow from independent reservoirs. One barrel was used to apply drug-free solution to enable rapid termination of drug application. Solution exchange measured by changes in open tip current was complete in 200 ms. However, complete exchange of solution around an intact cell was considerably slower (1s). Agonists were separately applied for 2s at 4min intervals, a time which was sufficient for responses to be reproducible. Estradiol was present for 4 min before and during the reapplication of agonists.

Chemicals

ATP, αβ-meATP, 17β-estradiol, 17α-estradiol and 17β-estradiol-BSA were all from Sigma, ICI 182,780 from Tocris. Solutions of ATP and other drugs were prepared in deionized water and stored frozen, except for ICI 182,780, which was dissolved in dimethylsulphoxide to 1mM. All drugs were then diluted in extracellular bathing solution to the final concentration.

Data analysis

Data are presented as mean ± S.E.M. Statistical analysis (Student's t test, F test) was performed using Excel (Microsoft, USA). A *P* value of less than 0.05 was considered as statistically significant.

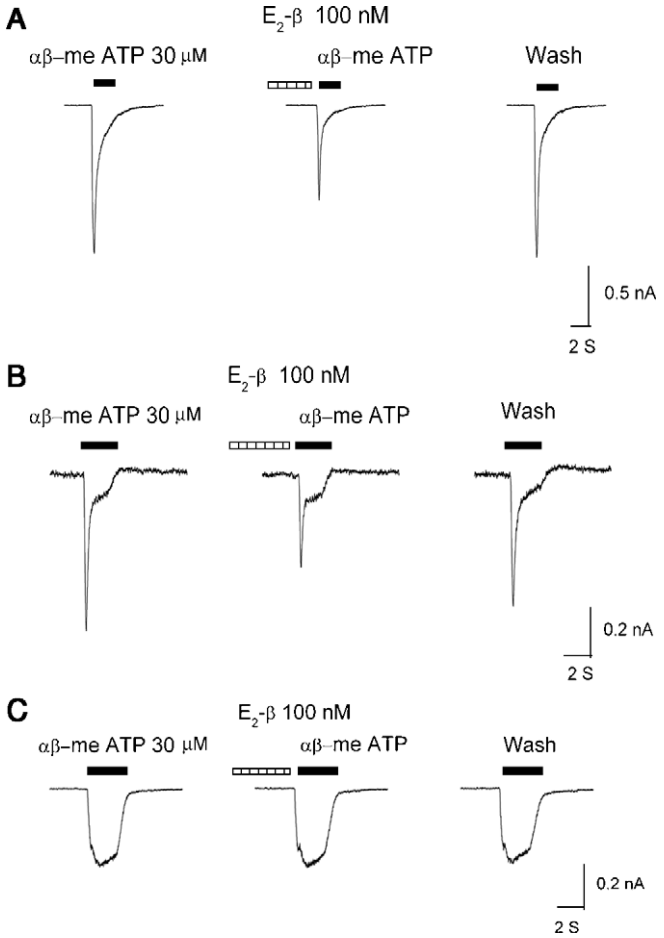

Fig. 1. Effects of 17β-estradiol on the amplitude of the currents evoked by αβ-meATP in DRG neurons. Representative traces show that inhibition by 17β-estradiol (100 nM) on the amplitude of transient current (A) and biphasic current (B) but not sustained current (C) evoked by αβ-meATP (30 μM). 17β-estradiol was preincubated for 4 min before co-applying with αβ-meATP.

Results

Currents evoked by αβ-meATP in DRG neurons

The dissociated DRG neurons were used between 3~30 hours of incubation and neurons with diameters of less than 25μm were chosen for investigation.

Following application of αβ-meATP (10 to 100μM, for 2 s), 45% (32/71) DRG neurons responded with a transient inward current (0.85 ± 0.01 nA), 20% (14/71) responded with a sustained current (0.90 ± 0.18 nA) and another 27% (19/71) cells responded with a biphasic current (1.07 ± 0.06 nA). In 6 (8%) cells, αβ-me ATP failed to induce a significant response. It is now clear that the transient response was mediated by homomeric $P2X_3$ receptors whereas the sustained response to αβ-me ATP was mediated by heteromeric $P2X_{2/3}$ receptors. The biphasic current was attributable to the presence of homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors in the same cell (Dunn et al., 2001, Pankratov et al., 2001).

The transient and biphasic currents evoked by αβ-meATP were reproducible for repeated challenges at 4 min intervals, with the variance of the peak less than 7%. The sustained currents evoked by αβ-meATP were reproducible at 3 min interval, with less than 10% rundown.

Rapid inhibition of 17β-estradiol on $I_{\alpha\beta\text{-}meATP}$

Application of 17β-estradiol alone, with a concentration up to 10 μM, did not evoke any detectable membrane currents in DRG neurons. In 13 out of 20 (65%) neurons that responded to

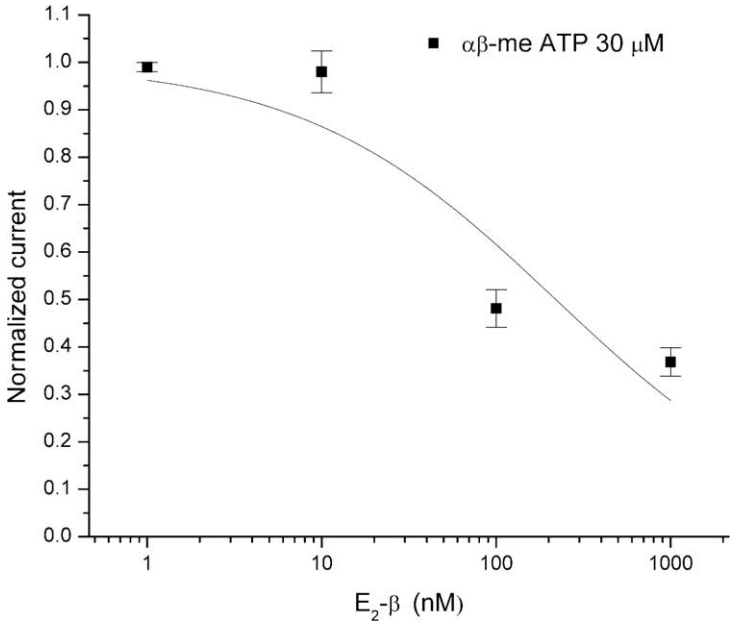

Fig. 2. Concentration-dependant inhibitory effect of 17β-estradiol on the transient current evoked by αβ-meATP (30 μM) in DRG neurons. Responses to αβ-meATP in the presence of 17β-estradiol were normalized with respect to the control current in the same neuron. Each data point represents mean ± S.E.M from 4–7 cells. The IC_{50} of the inhibitory effect of 17β-estradiol was estimated to be 219 ± 156 nM.

αβ-meATP (30μM) with a transient current, 17β-estradiol (100 nM, 4 min) resulted in a reduction of the current to 48 ± 4 % of control ($P < 0.05$, n = 8). An example of this effect is shown in Fig. 1A. When the concentration of 17β-estradiol was increased to 1000 nM, it led to a reduction of $I_{\alpha\beta\text{-meATP}}$ to 37 ± 3 % of control. Similarly, the transient component of biphasic responses evoked by αβ-meATP (30μM) was attenuated to 41 ± 4 % of control ($P < 0.05$, n=6) in the presence of 100 nM 17β-estradiol, as is illustrated in Fig. 1B. Full recovery of $I_{\alpha\beta\text{-meATP}}$ was observed following washing out 17β-estradiol. These observations indicate that 17β-estradiol inhibits the transient current evoked by αβ-meATP (30 μM) in DRG neurons in a concentration-dependant manner with an IC_{50} of 219 ± 156 nM (Fig. 2).

However, as is represented in Fig. 1C, 17β-estrodiol did not seem to affect the sustained currents induced by α,β-meATP. In 10 neurons that responded to α,β-meATP with a sustained current and 4 neurons that showed biphasic currents, $I_{\alpha\beta\text{-meATP}}$ after 4 min incubation with 17β-estrodiol (1000 nM) was 99 ± 1 % of control ($P > 0.05$). These observations suggest that 17β-estradiol selectively modulates $P2X_3$-mediated responses in DRG neurons.

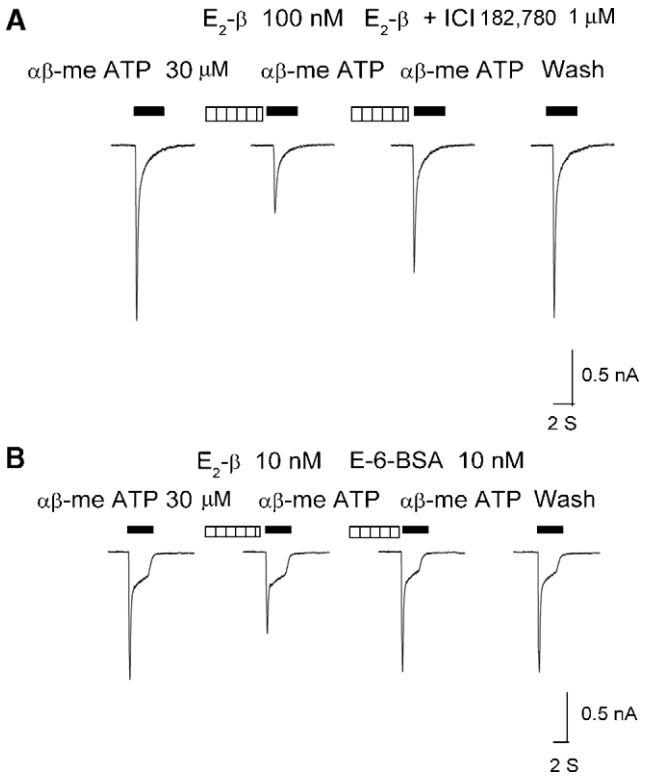

Fig. 3. The inhibitory effect of 17β-estradiol on $I_{\alpha,\beta\text{-meATP}}$ is blocked by ICI 182,780. (A), Representative traces showing that ICI 182,780 (1μM) blocks the inhibitory effect of 17β-estradiol on the transient currents evoked by αβ-meATP (30μM) in one DRG neuron; (B) A representative recording in which BSA-conjugated 17β-estradiol (E-6-BSA, 10nM) failed to affect the biphasic currents evoked by αβ-meATP whilst the non-conjugated 17β-estradiol inhibited this current in the same neuron.

Pharmacological profile of effects of 17β-estradiol

We then investigated the effects of ICI 182,780, a specific estrogen receptor antagonist, on the inhibitory action of 17β-estradiol on $I_{\alpha\beta\text{-meATP}}$. In the presence of ICI 182, 780 (1 μM), 17β-estradiol failed to affect $I_{\alpha\beta\text{-meATP}}$ (Figs. 3A and 4). Thus, $I_{\alpha\beta\text{-meATP}}$ following co-application of ICI 182,780 and 17β-estradiol was 88 ± 6 % of control (n=4, $P > 0.05$). Therefore the inhibitory effects of 17β-estradiol seemed to be mediated by ER_α or ER_β receptors.

To further exclude the possibility that the inhibitory effects of 17β-estradiol might be caused by alterations in the lipid composition of the plasma membrane as a result of the addition of the steroid, we tested the effects of 17α-estradiol, an inactive isoform of 17β-estradiol. At 100 nM, 17α-estradiol had no effect on $I_{\alpha\beta\text{-meATP}}$ in DRG neurons ($P > 0.05$, n=4) (Fig. 4).

To investigate whether 17β-estradiol acts on intracellular or membrane binding sites, we tested the effects of BSA-conjugated-17β-estradiol, which is unable to cross the plasma membrane. Following incubation with 17β-estradiol-BSA (10 nM), $I_{\alpha\beta\text{-meATP}}$ was 113 ± 1 % of control, which was not significantly different ($P > 0.05$, n = 6, Fig. 3B, Fig. 4).

Effect of 17β-estradiol on the ATP evoked sustained currents in rat nodose, SCG and OTG neurons

We then investigated the effect of 17β-estradiol on ATP currents (I_{ATP}) mediated by homomeric $P2X_2$ or heteromeric $P2X_{2/3}$ receptors in nodose ganglion, otic ganglion (OTG) and superior cervical ganglion

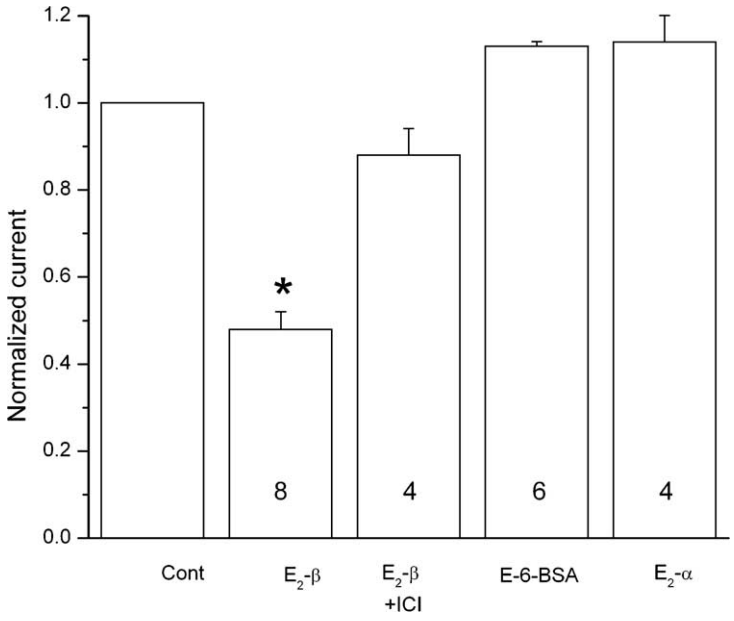

Fig. 4. The pharmacological profile of the effect of 17β-estradiol on $I_{\alpha\beta\text{-meATP}}$. The bar graph shows the normalized (% of control) whole-cell currents induced by α,β-meATP following various treatments. Addition of 17β-estradiol (100 nM, 4 min) significantly decreased transient $I_{\alpha\beta\text{-meATP}}$, whereas the inactive estradiol isomer, 17α-estradiol (100 nM, 4 min), and E-6-BSA (10 nM) had no effect. The selective estrogen receptor antagonist ICI 182, 780 (1 μM) blocked 17β-estradiol effect. The numbers inside each bar represent number of cells. *, $P < 0.05$.

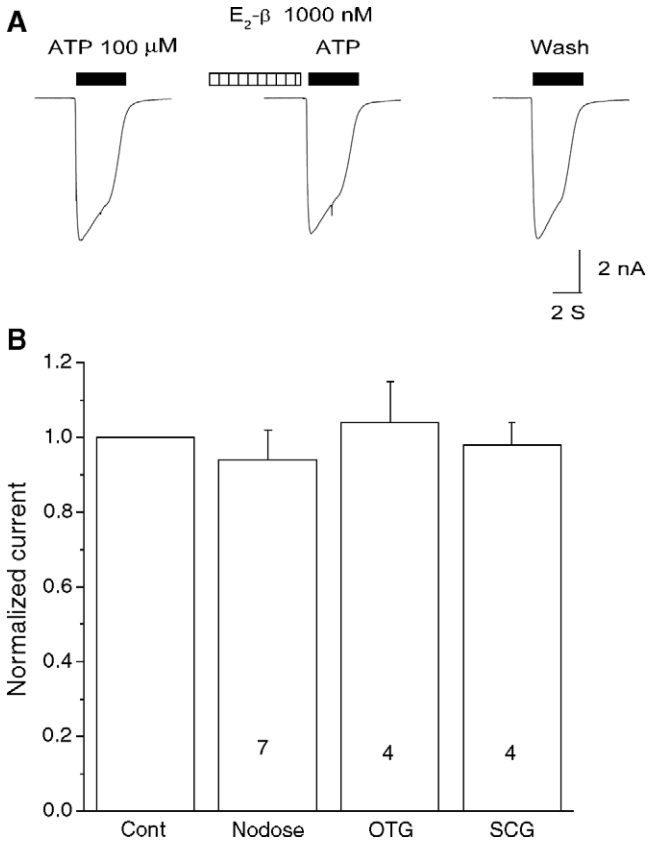

Fig. 5. The effect of 17β-estradiol on ATP-induced whole-cell currents in cells from three different ganglia. (A) Representative traces illustrating that 17β-estradiol (E_2-β, 1μM) had no effect on the sustained currents evoked by ATP (100 μM) in nodose neurons; (B) Summary data showing the effects of 17β-estradiol (E_2-β, 1μM)) on I_{ATP} in different ganglion neurons. Note that 17β-estradiol (E_2-β) attenuated transient I_{ATP} in DRG neurons but had no effect on the sustained I_{ATP} in nodose, SCG and OTG neurons. Values are mean ± S.E.M. The numbers in each bar represent the number of cells. *, $P < 0.05$.

(SCG) neurons. Seven out of 7 nodose neurons responded to 100 μM ATP with sustained currents. I_{ATP} following 4 min incubation with 17β-estradiol was 94 ± 8% of control, which was not significantly changed ($P > 0.05$, Fig. 5A and B). Four out of 4 OTG neurons responded to 100 μM ATP with sustained currents. In the presence of 17β-estradiol, I_{ATP} was 104 ± 11% of control ($P > 0.05$, Fig. 5B). Similarly, Four out of 4 SCG neurons responded to 100 μM ATP with sustained currents and I_{ATP} remained unaltered (98 ± 6% of control, $P > 0.05$) in the presence of 17β-estradiol (Fig. 5B).

Discussion

This study demonstrates that estradiol could rapidly modulate αβ-meATP induced transient currents in primary cultured DRG neurons but had no effect on the sustained response to the agonist. We found that this effect was stereospecific because equimolar concentration of the inactive estradiol isoform, 17α-estradiol, had no effect on $I_{\alpha\beta\text{-meATP}}$. Furthermore, estradiol effect was blocked by ICI 182,780.

Therefore, the effect of 17β-estradiol was not mediated by a nonspecific interaction with the plasma membrane and rather estradiol might interact with a receptor site with the classic intracellular estrogen receptor pharmacology. These results suggest that the female sex hormone can potentially modulate the actions of extracellular ATP.

ATP is present in virtually all cells and can be released by exocytosis or following cell lysis (Burnstock, 1996; Hamilton and McMahon, 2000) and extracellular ATP has a wide-spectrum of biological actions. ATP interacts with a family of ligand-gated ion channels, namely P2X receptors and a family of G-protein-coupled P2Y receptors. Previous evidence indicates that ATP may play an important role in nociception (Cook and McCleskey, 2002). DRG neurons express multiple P2 receptors, including $P2X_{1-6}$ and $P2Y_1$, $P2Y_4$ (Collo et al., 1996; Ruan and Burnstock, 2003). Three types of rapid responses, namely transient, sustained and biphasic current could be evoked by αβ-meATP in DRG neurons, as shown in this study. It is now clear that the transient current and the transient component of the biphasic current are mediated by homomeric $P2X_3$ receptor. The sustained current and the sustained component of the biphasic current were attributable to the activation of heteromeric $P2X_{2/3}$ receptors (Burgard et al., 1999; Dunn et al., 2000).

We found that 17β-estradiol inhibited the transient $I_{\alpha,\beta\text{-meATP}}$ in DRG neurons but had little effect on the sustained current, suggesting that 17β-estradiol effects vary across different P2X receptors. To further differentiate the selectivity of 17β-estradiol on different P2X subtypes, we tested its effects on ATP-induced currents in rat nodose, SCG and OTG cells, which are sensory, sympathetic and parasympathetic neurons, respectively. The sustained current evoked by ATP in nodose and OTG neurons has been reported to be attributed mainly to $P2X_{2/3}$ receptors with some additional $P2X_2$ receptors while the sustained current evoked by the same agonist in SCG neurons is believed to be mediated by $P2X_2$ receptors (Dunn et al., 2000). We found that the sustained I_{ATP} in these neurons were not affected by the addition of 17β-estradiol, which gave further support to the notion that the female sex hormone selectively modulates homomeric $P2X_3$-mediated events. It has been shown that $P2X_3$ receptor is localized in small- and medium-sized DRG neurons (Chen et al., 1995), which are predominantly nociceptors (Bradbury et al., 1998). Therefore, our results suggest that the female sex hormone can potentially play a role in pain by modulating $P2X_3$-mediated events in sensory neurons.

The actions of estrogen can potentially be mediated by a nongenomic as well as a genomic mechanism, depending on whether it binds to intracellular receptors and causes transcriptional activation. The effects of 17β-estradiol observed in this study occurred rather quickly (4 min), suggesting that the effects were mediated by a nongenomic rather than a genomic mechanism because at least 30 min are required for the genomic response to estrogen to occur (Orimo et al., 1993). E-6-BSA, a membrane impermeable construct, failed to mimic the effect of 17β-estradiol. These results can be explained by the estrogen acting on a receptor that is located entirely within the plasma membane bilayer and that the receptor perhaps does not express an extracellular domain (Mermelstein et al., 1996; Gu and Moss, 1998).

It has been reported that DRG neurons express both ER_α and ER_β (Sohrabji et al., 1994; Razandi et al., 1999). This study could not differentiate which of the two receptor subtypes mediated the inhibitory effects of 17β-estradiol on $I_{\alpha,\beta\text{-meATP}}$. However, it has been shown recently that 17β-estradiol attenuated ATP-induced Ca^{2+} influx in DRG neurons and the effect was attributable to membrane associated ER_α because the effect was not seen in DRG neurons from ER_α knock out (ER_αKO) mice (Chaban et al., 2003). Membrane-associated estrogen receptors have been linked to activation of both protein kinase A and protein kinase C (Razandi et al., 1999; Kelly and Levin, 2001; Nadal et al., 2001). The intracellular

signalling pathway that is involved in the rapid modulation of $P2X_3$-mediated responses in DRG neurons by 17β-estradiol awaits further investigation.

It has been reported that the plasma concentration of 17β-estradiol is less than 10 nM (Schlicher et al., 1998). The relative high concentrations of 17β-estradiol used in earlier (Zhang et al., 1994; Nakajima et al., 1995; Yamamoto, 1995; Kitazawa et al., 1997; Cario-Toumaniantz et al., 1998; Ruehlmann et al., 1998; Valverde et al., 1999) and in the present study (100 nM) suggest that experiments performed under these conditions are predominantly of pharmacological significance and the effect of estrogen in the physiological concentration remains unclear. However, given that sensitivity to noxious stimuli varies across the estrous (Frye et al., 1992; Martínez-Gómez et al., 1994; Kayser et al., 1996) and menstrual cycle (Giamberardino et al., 1997), it is pertinent to suggest, based on the current and previous evidence, that female sex hormones may modulate the sensitivity of primary afferent neurons to painful stimuli.

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References

Aloisi, A.M., Della Seta, D., Ceccarelli, I., Farabollini, F., 2001. Bisphenol-A differently affects estrogen receptors-alpha in estrous-cycling and lactating female rats. Neurosci Lett 310 (1), 49–52.

Bettini, E., Pollio, G., Santagati, S., Maggi, A., 1992. Estrogen receptor in rat brain: presence in the hippocampal formation. Neuroendocrinology 56 (4), 502–508.

Bradbury, E.J., Burnstock, G., McMahon, S.B., 1998. The expression of $P2X_3$ purinoreceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. Mol Cell Neurosci 12, 256–268.

Bradshaw, H.B., Temple, J.L., Wood, E., Berkley, K.J., 1999. Estrous variations in behavioral responses to vaginal and uterine distention in the rat. Pain 82 (2), 187–197.

Burgard, E.C., Niforatos, W., Vanbiesen, T., Lynch, K.J., Touma, E., Metzger, R.E., Kowaluk, E.A., Jarvis, M.F., 1999. P2X receptor-mediated ionic currents in dorsal root ganglion neurons. J. Neurophysiol. 82, 1590–1598.

Burnstock, G., 1996. A unifying purinergic hypothesis for the initiation of pain. Lancet 347, 1604–1605.

Burnstock, G., 2000. P2X receptors in sensory neurons. Br J Anesthesiol 84, 476–488.

Cairns, B.E., Sessle, B.J., Hu, J.W., 2001. Characteristics of glutamate-evoked temporomandibular joint afferent activity in the rat. J Neurophysiol 85 (6), 2446–2454.

Cario-Toumaniantz, C., Loirand, G., Ferrier, L., Pacaud, P., 1998. Non-genomic inhibition of human $P2X_7$ purinoceptor by 17beta-oestradiol. J Physiol 1 (508), 659–666.

Chaban, V.V., Mayer, E.A., Ennes, H.S., Micevych, P.E., 2003. Estradiol inhibits ATP-induced intracellular calcium concentration increase in dorsal root ganglia neurons. Neuroscience 118, 941–948.

Chen, C.C., Akopian, A.N., Sivilotti, L., Colquhoun, D., Burnstock, G., Wood, J.N., 1995. A P2X purinoreceptors expressed by a subset of sensory neurons. Nature 377, 428–431.

Collo, G., North, R.A., Kawashima, E., Merlo-Pich, E., Neidhart, S., Surprenant, A., Buell, G., 1996. Cloning of $P2X_5$ and $P2X_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. J Neurosci 16 (8), 2495–2507.

Cook, S.P., McCleskey, E.W., 2002. Cell damage excites nociceptors through release of cytosolic ATP. Pain 95 (1–2), 41–47.

Dawson-Basoa, M.E., Gintzler, A.R., 1998. Gestational and ovarian sex steroid antinociception: synergy between spinal and opioid system. Brain Res 757, 37–42.

Dunn, P.M., Liu, M., Zhong, Y., King, B.F., Burnstock, G., 2000. Diinosine pentaphosphate: an antagonist which discriminates between recombinant P2X(3) and P2X(2/3) receptors and between two P2X receptors in rat sensory neurones. Br J Pharmacol 130 (6), 1378–1384.

Dunn, P.N., Zhong, Y.u, Burnstock, G., 2001. P2X receptors in peripheral neurons. Prog Neurobiol 65, 107–134.

Eckersell, C.B., Popper, P., Micevych, P.E., 1998. Estrogen-induced alteration of mu-opioid receptor immunoreactivity in the medial preoptic nucleus and medial amygdala. J Neurosci 18, 3967–3976.

Frye, C.A., Bock, B.C., Kanarek, R.B., 1992. Hormonal milieu affects tailflick latency in female rats and may be attenuated by access to sucrose. Physiol Behav 52, 699–706.

Gintzler, A.R., Bohan, M.C., 1990. Pain thresholds are elevated during pseudopregnancy. Brain Res 507 (2), 312–316.

Giamberardino, M.A., Affaitati, G., Valente, R., Iezzi, S., Vecchiet, L., 1997. Changes in visceral pain reactivity as a function of estrous cycle in female rats with artificial ureteral calculosis. Brain Res 774, 234–238.

Gu, Q., Moss, R.L., 1998. Novel mechanism for non-genomic action of 17 beta-oestradiol on kainate-induced currents in isolated rat CA1 hippocampal neurones. J Physiol 506 (Pt 3), 745–754.

Hamilton, S.G., McMahon, S.B., 2000. ATP as a peripheral mediator of pain. J Auton Nerv Syst 81, 187–194.

Holdcroft, A., Sapsed-Byrne, S., Ma, D., Hammal, D., Forsling, M.L., 2000. Sex and oestrous cycle differences in visceromotor responses and vasopressin release in response to colonic distension in male and female rats anaesthetized with halothane. Br J Anaesth 85 (6), 907–910.

Holland, K.L., Norby, L.A., Micevych, P.E., 1998. Peripubertal ontogeny and estrogen stimulation of cholecystokinin and preproenkephalin mRNA in the rat hypothalamus and limbic system. J Comp Neurol 392, 48–57.

Kayser, V., Berkley, K.J., Keita, H., Gautron, M., Guilbaud, G., 1996. Estrous and sex variation in vocalization thresholds to hindpaw and tail pressure stimulation in the rat. Brain Res 742, 352–354.

Kelly, M.J., Lagrange, A.H., Wagner, E.J., Ronnekleiv, O.K., 1998. Rapid effects of estrogen to modulate G protein-coupled receptors via activation of protein kinase A and protein kinase C pathways. Steroids 64, 64–75.

Kelly, M.J., Levin, E.R., 2001. Rapid actions of plasma membrane estrogen receptors. Trends Endocrin Metab 4, 152–156.

Kim, Y.J., Hur, E.M., Park, T.J., Kim, K.T., 2000. Nongenomic inhibition of catecholamine secretion by 17-estradiol in PC12 cells. J Neurochem 6, 2490–2495.

Kitazawa, T., Hamada, E., Kitazawa, K., Gaznabi, A.K., 1997. Non-genomic mechanism of 17 beta-oestradiol-induced inhibition of contraction in mammalian vascular smooth muscle. J Physiol 499 (Pt 2), 497–511.

Martínez-Gómez, M., Cruz, Y., Salas, M., Hudson, R., Pacheco, P., 1994. Assessing pain threshold in the rat: changes with estrus and time of day. Physiol Behav 55, 651–657.

Mermelstein, P.G., Becker, J.B., Surmeier, D.J., 1996. Estradiol reduces calcium current in rat neostratial neurons via a membrane receptor. J Neurosci 2, 595–604.

Nadal, A., Diaz, M., Valverde, M., 2001. The estrogen trinity: membrane, cytosolic, and nuclear effects. News in Physiol Sci 16, 251–256.

Nakajima, T., Kitazawa, T., Hamada, E., Hazama, H., Omata, M., Kurachi, Y., 1995. 17beta-Estradiol inhibits the voltage-dependent L-type Ca2+ currents in aortic smooth muscle cells. Eur J Pharmacol 294 (2–3), 625–635.

Orimo, A., Inoue, S., Ikegami, A., Hosoi, T., Akishita, M., Ouchi, Y., Muramatsu, M., Orimo, H., 1993. Vascular smooth muscle cells as target for estrogen. Biochem Biophys Res Commun 195 (2), 730–736.

Pankratov, Yu.V., Lalo, U.V., Dashkin, A.N., Krishtal, A., 2001. Heterogeneity of the functional expression of P2X3 and P2X2/3 receptors in the primary nociceptive neurons of rat. Neurochem Res 26 (8–9), 993–1000.

Papka, R.E., Srinivasan, B., Miller, K.E., Hayashi, S., 1997. Localization of estrogen receptor protein and estrogen receptor messenger RNA in peripheral autonomic and sensory neurons. Neuroscience 79 (4), 1153–1163.

Pappas, T.C., Gametchu, B., Watson, C.S., 1995. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. FASEB J 9, 404–410.

Priest, C.A., Eckresell, C.B., Micevych, P.E., 1995. Estrogen regulates preproenkephalin-A mRNA levels in the rat ventromedial nucleus: temporal and cellular aspects. Brain Res Mol Brain Res 28, 251–262.

Razandi, M., Pedram, A., Greene, G., Levin, E., 1999. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcripts: studies of ERα and ERβ expressed in Chinese hamster ovary cells. Mol Endocrinol 13, 307–319.

Robbins, A., Berkley, K.J., Sato, Y., 1992. Estrous cycle variation of afferent fibers supplying reproductive organs in the female rat. Brain Res 596 (1–2), 353–356.

Ruan, H.Z., Burnstock, G., 2003. Localisation of P2Y1 and P2Y4 receptors in dorsal root, nodose and trigeminal ganglia of the rat. Histochem Cell Biol 120 (5), 415–426.

Ruehlmann, D.O., Steinert, J.R., Valverde, M.A., Jacob, R., Mann, G.E., 1998. Environmental estrogenic pollutants induce acute vascular relaxation by inhibiting L-type Ca2+ channels in smooth muscle cells. FASEB J. 12 (7), 613–619.

Sapsed-Byrne, S., Ma, D., Ridout, D., Holdcroft, A., 1996. Estrous cycle phase variations in visceromotor and cardiovascular responses to colonic distension in the anesthetized rat. Brain Res 742 (1–2), 10–16.

Schlicher, F., Fauber, U., Louton, T., Schunak, T., 1998. Tissue distribution of sex steroids: concentration of 17beta-oestradiol and cyproterone acetate in selected organs of female Wistar rats. Pharmacol Toxicol 82, 34–39.

Sinchak, K., Micevych, P.E., 2001. Progesterone blockade of estrogen activation of μ-opioid receptors regulates reproductive behavior. J Neurosci 21, 5723–5729.

Sohrabji, F., Miranda, R.C., Toran-Allerand, C.D., 1994. Estrogen differentially regulates estrogen and nerve growth factor receptor mRNAs in adult sensory neurons. J Neurosci 14, 459–471.

Suzuki, N., Hardebo, J.E., 1991. The pathway of parasympathetic nerve fibres to cerebral vessels from the otic ganglion in the rat. J Auto Nervous System 36 (1), 39–46.

Taleghany, N., Sarajari, S., DonCarlos, L.L., Gollapudi, L., Oblinger, M.M., 1999. Differential expression of estrogen receptor alpha and beta in rat dorsal root ganglion neurons. J Neurosci Res 57, 603–615.

Valverde, M.A., Rojas, P., Amigo, J., Cosmelli, D., Orio, P., Bahamonde, M.I., Mann, G.E., Vergara, C., Latorre, R., 1999. Acute activation of Maxi-K channels (hSlo) by estradiol binding to the beta subunit. Science 285 (5435), 1929–1931.

Yamamoto, T., 1995. Effects of estrogens on Ca channels in myometrial cells isolated from pregnant rats. Am J Physiol 268 (1 Pt 1), C64–C69.

Zhang, F., Ram, J.L., Standley, P.R., Sowers, J.R., 1994. 17 beta-Estradiol attenuates voltage-dependent Ca^{2+} currents in A7r5 vascular smooth muscle cell line. Am J Physiol 266 (4 Pt 1), C975–C980.