

CELL MEMBRANE GPER AND ER α ON MYOMETRIUM: TWO DISTINCT RECEPTORS WITH DISTINCT SIGNALLING PATHWAYS

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Abstract

The “rapid” effects of oestrogens on myometrium mainly result by activating cell membrane oestrogen receptors: ER α and GPER (G protein coupled oestrogen receptor 1). In contrast to the contractile effect induced by G-1 (GPER agonist), effect involving the opening of L-type calcium channels, oestradiol (E₂) inhibited until arrest the spontaneous contractile activity and significantly decreased the contraction induced by high K⁺ or oxytocin. The effects of E₂ were not blocked by G-15 (GPER antagonist). We concluded that the “rapid” effects E₂-induced on myometrium are the result of cell membrane ER α activation and mainly consist of the inhibition of L-type calcium channels. It is a sticking difference between the genomic and non-genomic effect of E₂ on oxytocin-induced signalling pathway. Finally, it is very probable a masking/inhibition (modulator) effect of ER α on cell membrane GPER activity.

Rezumat

Efectele de tip „rapid” ale estrogenilor la nivelul miometrului sunt, aproape exclusiv, rezultatul activării unor receptori specifici membranari: ER α și GPER (*G protein coupled estrogen receptor 1*). În contrast cu efectul ocitocic indus de G-1 (agonist specific GPER), efect datorat, în special, deschiderii canalelor membranare de calciu tip L, estradiolul (E₂), în aceleași concentrații, blochează complet activitatea spontană a fibrelor musculare uterine și diminuează semnificativ contracțiile induse de KCl 60 mM sau de oxitocină. Aceste efecte nu sunt afectate de G-15 (antagonist specific GPER). Datele noastre demonstrează ca efectele de tip “rapid” ale E₂ asupra miometrului sunt rezultatul activării ER α membranari și se datorează, în principal, diminuării permeabilității canalelor membranare de calciu de tip L. De asemenea, se poate constata că aceste efecte de tip non-genomic sunt complet opuse față de cele “clasice” induse de E₂ asupra mușchiului uterin prin mecanism genomic. De asemenea, se poate concluziona ca ER α posedă un efect de tip modulator asupra activității GPER la nivel miometrial.

Keywords: oestradiol, G protein coupled oestrogen receptor 1 (GPER), ER α , L-calcium channels, oxytocin

Introduction

Oestrogens are steroid hormones involved in the control and regulation of reproduction, growth, development, cognition, behaviour, cardiovascular and immune functions. Their effects are the result of binding to specific oestrogen receptors (ERs): ER α [8], ER β [10] and GPER (G protein-coupled oestrogen receptor 1 [2, 3]), followed by the initiation of two types of intracellular signalling pathways: genomic (nuclear) and non-genomic (non-nuclear). The first assume the modulation of different transcriptional processes and represent the

“slow” but the most important mechanism of action. The second-type is responsible for the “rapid” oestrogenic effects and involve the generation of second messengers: Ca²⁺, cAMP, and NO, as well as activation of receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF-1R), and/or protein/lipid kinases (PI 3-kinase, protein kinase B (Akt), mitogen activated protein kinase (MAPK) family members, Src family members, and protein kinase A/C (PKA/PKC)) [5-7, 9, 11, 14].

On myometrium, oestrogens (oestradiol – E₂ being the most active) induce hypertrophic and hyperplastic effects, mainly as a result of nuclear ER α binding (the expression of ER β is far lower [16]). The hypertrophy especially affects structures involved in the increase of the uterine smooth muscle contractility and excitability of (receptors, ion-channels, signalling pathways, contractile proteins, energy substrate etc. [13]). The “rapid” effects are far less known and occur after E₂ binding to non-nuclear ER α and GPER. It is already proven the presence of ERs on cell membrane, mitochondria and endoplasmatic reticulum [12], but the physiological role of the last two locations is still completely unclear.

We previously published important data about the presence of GPER and its possible involvement in rat uterine contraction [15]. In this paper we compared the effects obtained by separate activation of membrane ER α with those resulted by independent GPER stimulation.

Materials and Methods

The non-pregnant myometrium was obtained from female Sprague-Dawley rats, weighing 180-200 g. The animals were kept in cages, with 8 hours of light/day rhythm, with permanent water supply and with a normal diet, established by the Nutrition and Epidemiology Departments.

After anaesthesia with thiopental sodium 1 g/kgbw the two uterine horns from each animal were rapidly excised.

All experiments were performed under the American University Laboratory Animal Care Committee Agreement and were approved by the University Ethics Committee.

The two uterine horns from each animal were introduced in Krebs solution, with the following composition (mM): 127 NaCl, 1.9 KCl, 1.2 KH₂PO₄, 2.4 CaCl₂, 1.3 MgCl₂, 26 NaHCO₃ and 5 glucose), oxygenated with a mixture of 95% O₂ and 5% CO₂, thermostated at 37°C and then cut into strips of 3 mm length.

Each muscular fragment was introduced into a 5 mL organ bath and connected to a force transducer

(ML T0201/RAD; ADInstruments, Colorado Springs, CO, USA) coupled to a Quad Bridge Amplifier (ADInstruments.). Contractions were recorded using a PowerLab system and Chart 6 software (ADInstruments). The resting load was adjusted to 0.5 g.

After equilibration, the contractile effect was quantified by 3 parameters:

Area under the contractility curve. It was measured as the integrated force for 10 min before administration and after the beginning of the effect induced by specific compounds.

Frequency of myometrial contractions, as the number of spontaneous contractions in a period of 10 min before and after the application of the compound.

Amplitude of myometrial contractions. The amplitude of myometrial contractions was defined as the distance between the peak and the initial baseline of the contractions in a period of 10 min before and after the administration of each compound.

Statistical analysis

The non-parametric Mann-Whitney test (MW) was used; a p value < 0.05 was considered statistically significant, while a p value < 0.001 was considered highly significant.

Chemicals

The experiments were performed using β -oestradiol (Sigma-Aldrich), G-1 (Sunset Molecular), G-15 (Sunset Molecular), and oxytocin (Sigma-Aldrich).

Results and Discussion

In basal conditions, the area under the contractility curve (AUC) was 92.41 ± 14.96 g.s. / 10 min, the frequency of myometrial contractions was 5.30 ± 0.93 contractions / 10 min, and the amplitude of myometrial contractions was 1.01 ± 0.07 g (n = 17). Administration of E₂ (5×10^{-7} M) induced a time dependent decrease in frequency and amplitude of spontaneous contractions as well as by area under contractility curve and finally abolished the spontaneous activity after approx. 10 min (a representative trace is shown in Figure 1.A.).

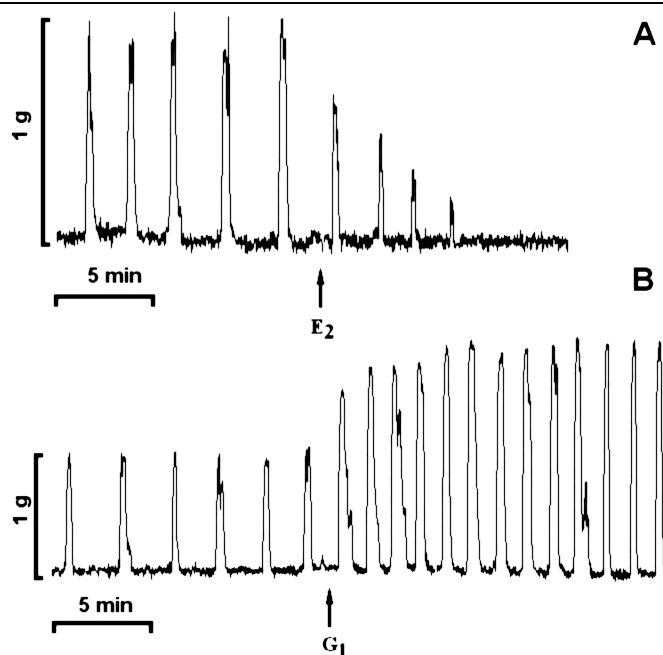


Figure 1.

Effects of Oestradiol (E_2) and G-1 on the contractility of rat uterine strips. In basal conditions, the area under the contractility curve was 92.41 ± 14.96 g.s / 10 min, the frequency of myometrial contractions was 5.30 ± 0.93 contractions / 10 min, and the amplitude of myometrial contractions was 1.01 ± 0.07 g (n = 17).

A. E_2 (5×10^{-7} M) progressively diminished and finally abolished the spontaneous uterine contractility.

B. G-1 (5×10^{-7} M) (a specific GPER agonist) caused a robust effect on uterine strips contractility.

Meanwhile, KCl 60 mM induced a sustained contraction, whose AUC increased to 623.43 ± 91.07 % (n = 7) - $p < 0.001$. A second administration of KCl 60 mM (after 3 wash-outs, every 10 min) increased the area under the contractility curve to 612.57 ± 87.44 % (n = 7), value almost equal with that obtained to the first

administration - $p > 0.05$ (data not shown). After pre-treatment with E_2 (5×10^{-7} M, 5 min), KCl 60 mM induced a contraction with an AUC increased only by 307.29 ± 51.15 % (n = 7) - $p < 0.001$, when compared to KCl alone (a representative trace is shown in Figure 2).

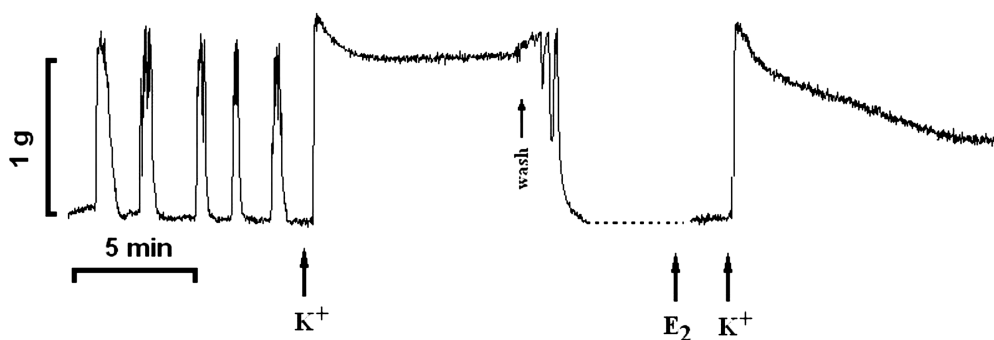


Figure 2.

Effect of E_2 on high K^+ -induced rat myometrial contraction. KCl 60mM increased the area under the contractile wave (AUC) by 623.43 ± 91.07 % (n = 7). A second administration of KCl 60 mM (after 3 wash-outs, every 10 min) increased the area under the contractility curve by 612.57 ± 87.44 % (n = 7), value almost equal with that obtained to the first administration (data not shown). After pre-treatment with E_2 (5×10^{-7} M, 5 min), the second administration of KCl 60 mM induced a contraction with an AUC increased only by 307.29 ± 51.15 % (n = 9).

As we previously reported, administration of G-1 (5×10^{-7} M) (a specific GPER agonist) caused a robust effect on uterine strips contractility (a representative trace is shown in Figure 1.B.), and

this effect was almost completely abolished by prior administration of G-15 (a specific GPER antagonist; 5×10^{-6} M; 15 min) or nifedipine (5×10^{-7} M, 10 min) [15].

Oxytocin (10^{-7} M) induced a contraction with an area under the contractility curve measuring 996.33 ± 122.14 g.s. / 10 min ($n = 9$), which means an increase by 147% compared with that induced by a

previous administration of KCl 60 mM - $p < 0.001$ (followed by 3 wash-outs every 10 min – a representative trace is shown in Figure 3A.).

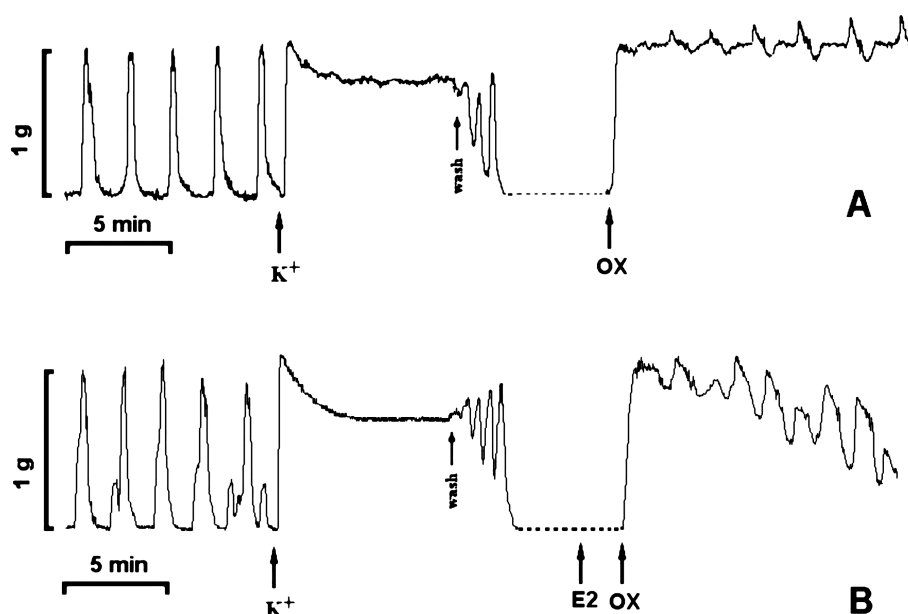


Figure 3.

Effect of E_2 on oxytocin-induced rat uterine contraction.

A. Oxytocin (10^{-7} M) induced a contraction with an area under the contractility curve measuring 996.33 ± 122.14 g.s. / 10 min ($n = 9$), which means an increase by 147% compared with that induced by a previous administration of KCl 60mM (followed by 3 wash-outs every 10 min).

B. After E_2 administration (5×10^{-7} M, 5 min), the contraction oxytocin-induced was only 468.22 ± 72.03 g.s. / 10 min ($n = 9$).

After E_2 administration (5×10^{-7} M, 5 min), the contraction oxytocin-induced was only 468.22 ± 72.03 g.s. / 10 min ($n = 9$) - $p < 0.001$, when compared to oxytocin alone (a representative trace is shown in Figure 3.B.).

Instead, the pre-administration of G-1 (5×10^{-7} M, 5 min) did not affect the contractile effect of oxytocin (data not shown).

Conclusions

We can conclude that the “rapid” effects of oestrogens (mainly E_2) on myometrium are almost completely the result of cell membrane $ER\alpha$ and GPER activation.

It was established that although all 3 types of oestrogen receptors: $ER\alpha$, $ER\beta$ and GPER are present in myometrium; $ER\beta$ is far less expressed [16]. Furthermore, extranuclear localization of $ER\alpha$ and GPER are mainly in cell membrane, the mitochondrial and endoplasmic reticulum oestrogen receptors have not proven a biological role until date.

We previously reported important data about the presence of GPER in rat myometrium and its roles

on rat uterine contraction and we showed that G-1 induced a sustained contractile effect [15]. This effect was completely abolished by G-15 and nifedipine (a L-type calcium blocker), proving the activation of L-type calcium channels by stimulated GPER.

The G-1-induced contraction of uterine strips is in sharp contrast with data reported for E_2 , which activates non-selectively both $ER\alpha$ and GPER [4]. Oestradiol inhibited until complete arrest the spontaneous contractile activity.

Previous studies indicate that prolonged exposure (days) to E_2 enhances Ca^{2+} entry in resting and K^+ -stimulated myometrium, effect probably due by an increase in Ca^{2+} channel density [1]. Moreover, chronic administration of E_2 induced an increase in ADPR cyclase, enzyme in charge to generate cyclic ADP ribose, a well-established Ca^{2+} mobilizing second messenger [3].

In contrast, our data is showing that acute exposure to E_2 inhibits the calcium entry through L-type channels opened by high K^+ -induced depolarisation. This effect was not influenced by G-15, proving that the effect of E_2 is solely due to $ER\alpha$ activation.

We can conclude that the strikingly different effects on contractility between E_2 and G-1 is the result of the opposite effects on calcium signalling pathways, the $ER\alpha$ -induced inhibition on L-type calcium channels being dominant.

Also it is possible to exist a masking / inhibition of GPER activity by activated $ER\alpha$, as consequence of a direct modulator effect of $ER\alpha$ on cell membrane GPER.

Finally, it is very probable that the inhibition of oxytocin-induced contraction by activated $ER\alpha$ is also the result of the decrease in L-type calcium channels conductance, but the inhibition of other entities belonging to the oxytocin-dependent pathway also cannot be excluded.

It is very interesting that oestrogens, by the "slow" genomic mechanism increase both structurally and functionally all the signalling complex oxytocin-dependent but by "rapid" extranuclear effect they are able to inhibit the activity of the same complex.

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