

# The Estrogen-Induced Effects on Myometrium Are the Result of Activation of Two Different Receptor Types: GPER and ER $\alpha$

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**ABSTRACT:** GPER (G protein coupled estrogen receptor 1), a particular estrogen binding site, is ubiquitously present in human tissues, but its precise physiological role is still very disputed. GPER is associated with normal and abnormal estrogen-dependent proliferations in female tissues and is involved in generation of rapid estrogenic answers. A very important fact is that GPER-induced genomic effects are additive to those mediated by “classic” estrogen receptors, but regarding the rapid effects, as we prove in this study, these can be significant different or even antagonistic.

**KEYWORDS:** GPER, ER $\alpha$ , myometrium, contraction, G1

## Introduction

GPER was firstly cloned by Owen and col. in 1996 [1] who called it GPR30. He described its particular structure, completely different to that of “classic” estrogen receptors (ERs), this estrogen binding site belonging to the big family of transmembranal receptors functionally coupled with G proteins (Fig. 1.). Few years after (2000), Filardo and col. proved its affinity for estrogens and renamed it GPER [2]. To date,

several studies showed the generation of different second messengers as well as activation of some kinases following GPER-stimulation. Also, there are reports about its higher density in abnormal proliferations estrogen-dependent in different types of female tissues.

In this study we compared the “rapid” effects GPER-induced with those resulted by ERs (especially ER $\alpha$ ) activation on myometrium.

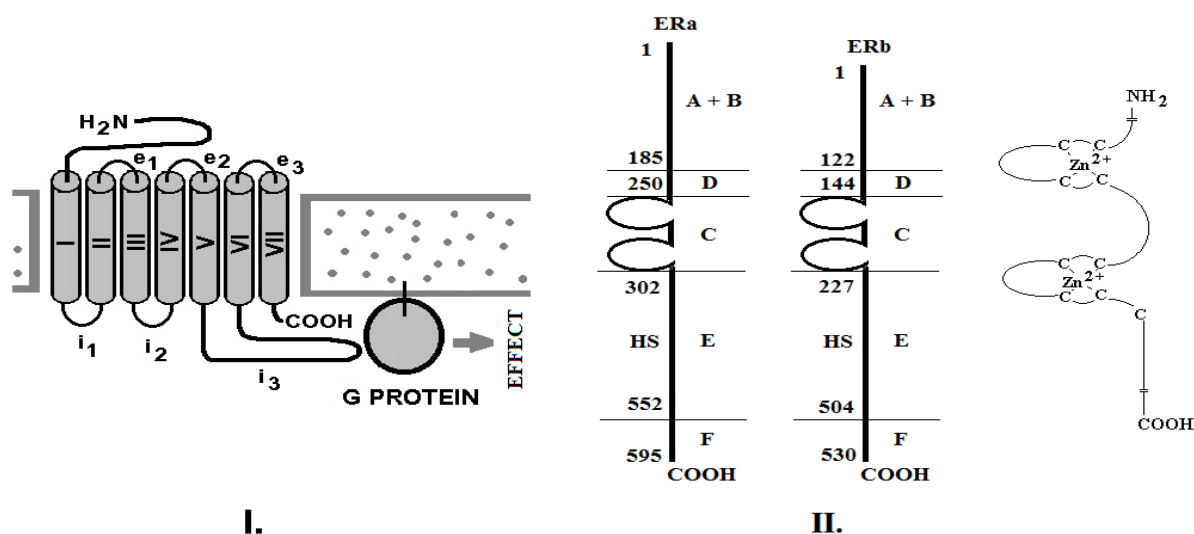


Figure 1. Structure of I. GPER and II. “classic” estrogen receptors: ER $\alpha$  and ER $\beta$ . The DNA binding site of the “classic” estrogen receptors. is detailed

## Material and method

We used female Sprague-Dawley rats, weighting 180-200g. The protocol was approved by the Ethical Committee of the University of Medicine and Pharmacy of Craiova and was similar with that previously reported, with some modifications [3].

Briefly, after put to sleep with tiopental, the animals were rapid sacrificed and the two uterine horns were yielded and cut into slices of 2mm long.

The uterine fragments were introduced in a 5-ml organ bath in Krebs-Henseleit solution with the following composition: (mM): 127 NaCl, 1.9 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 2.4 CaCl<sub>2</sub>, 1.3 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub> and 5 glucose, oxygenated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> and thermostated at 37°C . The strips were pretensioned to 0.5g. The contractile activity was measured with a force transducer (ML T0201/RAD; ADInstruments, Colorado Springs, CO, USA) coupled to a Quad Bridge Amplifier (ADInstruments,) and recorded using a PowerLab system and Chart 6 software (ADInstruments). We measured the frequency, the mean amplitude and the area under the contractility curve.

After a 10min. period of stabilization, the uterine fragments were washed with Krebs solution and the spontaneous activity was recorded during another 10min. This recording was used as control.

After 10min the uterine fragments were washed with Krebs solution and after 5min G1

(a specific GPER agonist) was added in following concentrations: 5.10<sup>-9</sup>M, 5.10<sup>-8</sup>M and 5.10<sup>-7</sup>M.

These experiments were redone by firstly introducing in the organ bath G15 5.10<sup>-6</sup>M (a specific GPER antagonist).

The second type of determinations was performed using estradiol (E<sub>2</sub>) in the same concentrations: 5.10<sup>-9</sup>M, 5.10<sup>-8</sup>M and 5.10<sup>-7</sup>M, with and without G15 5.10<sup>-6</sup>M.

The data was analyzed using one-way ANOVA test, p<0.05 being considered statistically significant and p<0.01 high statistically significant.

We used β-estradiol (Sigma-Aldrich), respectively G-1 and G-15 (Sunset Molecular).

## Results

The basal contractile activity recorded had the following characteristics: the area under the contractility curve was 92.41±14.96 g.s/10 min., the frequency was 5.30±0.93 contractions/10 min., and the mean amplitude of myometrial contractions was 1.01±0.07g

G1 increased the contractile activity in a concentration-dependent manner (Tab. I).

Its effects were completely abolished by G15 5.10<sup>-6</sup>M (Fig. 2.).

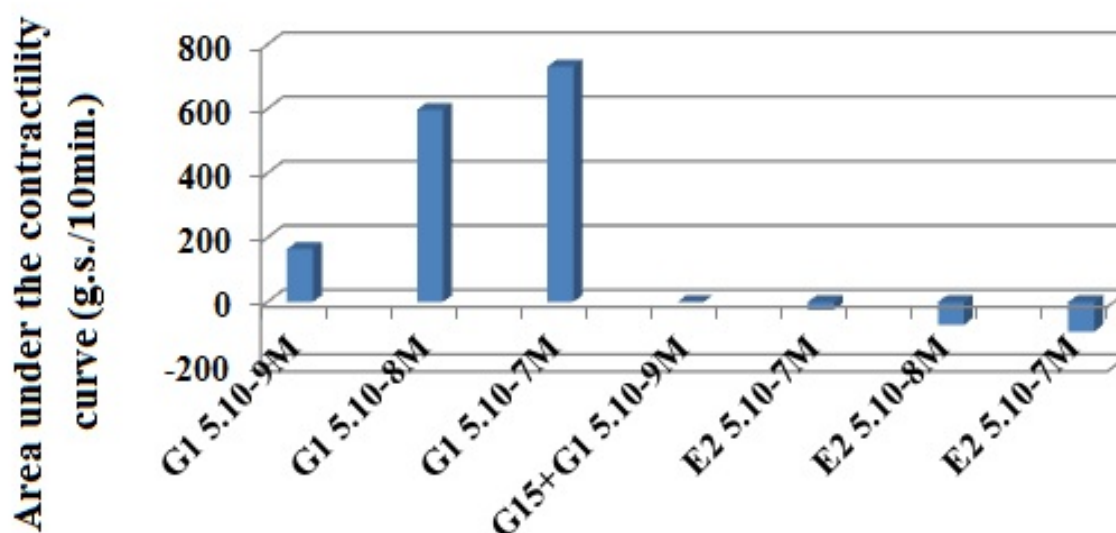
E<sub>2</sub> decreased the spontaneous uterine activity also in a concentration-dependent manner, 5.10<sup>-7</sup>M finally arresting the automatism (Tab. I.).

This effect was not altered by prior administration of G15 5.10<sup>-6</sup>M (data not shown).

**Table I. The characteristics of spontaneous, respectively of different concentrations of G1 and E2-induced effects on myometrial strips contractility (l=2mm)**

Contractil activity	Frequency (/10min.)	Mean amplitude (g)	Area under the curve (g.s./10min)
Spontaneous (n=11)	5.30 ± 0.93*	1.01 ± 0.07**	92.41 ± 14.96*
G1 5.10 <sup>-9</sup> M (n=7)	8.19 ± 0.72**	1.12 ± 0.11*	164.39 ± 21.62**
G1 5.10 <sup>-8</sup> M (n=5)	19.28 ± 0.1.87*	1.52 ± 0.14*	597.37± 7.29*
G1 5.10 <sup>-7</sup> M (n=9)	21.32± 2.39*	1.65± 0.39*	731.25 ± 21.67*
E2 5.10 <sup>-9</sup> M (n=7)	3.68 ± 0.81*	0.83 ± 0.12**	67.93 ± 10.84*
E2 5.10 <sup>-8</sup> M (n=8)	1.57 ± 0.38**	0.29 ± 0.09*	21.15 ± 8.35*
E2 5.10 <sup>-7</sup> M (n=5)	0	0	0

n=number of experiments; \* - p<0.05; \*\* - p<0.01



**Figure 2.** The area under the contractility curve (AUC) for spontaneous, respectively for different concentrations of G1 and E<sub>2</sub>-induced effects on uterine fragments activity (l-2mm)  
G15 5.10<sup>6</sup>M abolish the contraction G1 5.10<sup>7</sup>M-induced

## Discussions

GPER-activation is followed by generation of second messengers (Ca<sup>2+</sup>, NO, cAMP) and activation of several tyrosine/protein kinases, these last ones being involved in normal and abnormal tissue proliferation [4,5,6,7,8,9].

The cell growth signaling pathways GPER-dependent generate similar effects with those resulted by ERs genomic mechanism of action, but the “rapid” pathways induce completely different effects.

In female human tissues ERs are represented almost exclusively by ER $\alpha$ , ER $\beta$  being far less expressed [10]. ER $\alpha$  is specifically activated by E<sub>2</sub>.

As we previously reported, ER $\alpha$  but not GPER, inhibit L-calcium channels in myometrium and E<sub>2</sub> binds ER $\alpha$ , effect followed by inhibiting/masking the concomitant non-selectively activation of GPER [11].

In this study we demonstrated that ER $\alpha$ -activation E<sub>2</sub>-induced diminish until complete arrest the spontaneous uterine activity in a concentration-dependent manner.

Opposite, GPER activation increase the myometrial contractility also in a concentration-dependent manner.

These antagonistic effects are probably the result of different type of effects on L-calcium channels, but can be involved also other signaling pathways (cAMP etc).

## Conclusions

On myometrium, the “rapid” effects GPER-induced are opposite to those of ER $\alpha$ -induced and, as previously reported, this is at least the result of the inhibiting of calcium conductance through cell membrane L-type channels. Also, E<sub>2</sub>, after binding to

ER $\alpha$ , probably blocks the nonspecifically GPER-activation.

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All authors contributed equally to this paper.

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