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Genistein and daidzein modulate in vitro rat uterine contractile activity

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Abstract

The present study investigated the effect of genistein, daidzein and estradiol on in vitro rat uterine responsiveness to oxytocin (OT) and $PGF_2\alpha$ or luprostiol (L). In a first experiment, animals were either sham-operated (SH; $n = 5$), or ovariectomized (OVX; $n = 20$) and orally treated for three months with either genistein (G; $n = 5$; 10 μg/g BW/d) or daidzein (D; $n = 5$; 10 μg/g BW/d) or 17α-ethinylestradiol (E; $n = 5$; 23 μg/kg BW/d) or untreated (OVX; $n = 5$). At necropsy, the basal uterine tension was lower in OVX, G and D than in SH, the highest value being measured in E. Oxytocin (10^{-12}; 10^{-11} M) or $PGF_2\alpha$ (10^{-12}; 10^{-9} M) induced an increase in SH, but not in OVX, E and G. In D, only the highest doses were efficient. In a second experiment, 20 intact animals were s.c. injected with either genistein (G; $n = 5$; 10 μg/g BW) or daidzein (D; $n = 5$; 10 μg/g BW) or estradiol benzoate (E; $n = 5$; 23 μg/kg BW) or vehicle (C: controls; $n = 5$), and killed 24 h later. In C and E, OT (10^{-15} to 10^{-10} M) or L (10^{-12} to 10^{-7} M) stimulated uterine contractile activity in a dose-dependent manner until a maximal level. On the opposite, in G and D, contractile agents (except the highest luprostiol doses) did not stimulate myometrium contractions. Moreover, radioligand binding assays showed that genistein or daidzein inhibited the specific binding of [^{3}H] estradiol to the calf uterus estrogen receptor (ER). Therefore, it could be postulated that both genistein and daidzein might bind to the rat uterus ER, inducing either anti-estrogenic or very weak estrogenic effects (depending on the experimental conditions) on in vitro uterine responsiveness to OT and $PGF_2\alpha$ or luprostiol.

Keywords: Genistein; Daidzein; Uterine contractile activity; Rat

1. Introduction

Uterine muscular activity is influenced by ovarian steroid hormones, nervous activity, and endogenous humoral or locally produced uterus-activating agents such as the neurohypophyseal peptides, the prostaglandins, and various biogenic amines [1]. Severe developmental and reproductive disorders in wild animals have been linked to high exposure to persistent environmental chemicals with hormonal activity [2]. Sheep grazing on estrogenic pastures exhibited reproductive failures, which were largely attributed to phytoestrogen effects on uterus and cervix [3]. The 'phytoestrogen' term comes from the estrogen-like structure of these plant molecules which possess two hydroxyl groups (one in the aromatic A ring and the other at the opposite end of the molecule) separated by a similar distance. As a result, they can bind to the estrogen receptor (ER), although their occupancy time and affinity for the receptor are reduced compared to estradiol [4]. Broadly defined, phytoestrogens include isoflavones, coumestans and lignans, found mainly in soybeans, clover or alfalfa sprouts, and oilseeds such as flaxseed, respectively [5]. Genistin and daidzin, and their biologically active aglycon forms, genistein and daidzein, are the major isoflavones in soybean foods [6].

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The classical action of phytoestrogens (using genistein as the model molecule) on ER consists of several steps: combination with ER in the cytosol, movement of the complex into the nucleus for activation of estrogen response elements (ERE) after dimerization with one of several possible molecules, stimulation of DNA-directed mRNA synthesis, and production of new protein molecules which are specific to the tissue-cell type [7]. Soybean isoflavones are under intensive investigation because plasma phytoestrogen concentrations in populations consuming regularly soybean products are maintained at high levels [8], and because soybean consumption might be associated with potential health benefits such as prevention of atherosclerosis progression, bone preservation, lowering of cancer risks, and positive effects on cognitive function, dementia, hot flushes and vaginal symptoms in postmenopausal women. In rats, although soybean estrogens do not demonstrate any estrogenic activity for vaginal cytology or uterine weight [9], opposite effects were reported in ovariectomized rats receiving daidzin (but not genistin) [10] or genistein [11].

Therefore, the present study investigated the effect of genistein or daidzein on in vitro rat uterine responsiveness to oxytocin (OT) and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) or luprostiol (L). Additionally, the genistein or daidzein affinity for the calf uterus ER was also determined.

2. Materials and methods

2.1. Uterine responsiveness to oxytocin and $PGF_2\alpha$ or luprostiol in phytoestrogen-treated rats

2.1.1. Animals and treatments

Two series of experiments were performed in accordance with current legislation on animal experiments in France. Forty-five 12-month-old virgin female Wistar rats were purchased from I.N.R.A. (Clermont-Ferrand/Theix, France) and housed individually at 21 ± 1°C, with 12/12 h light/dark cycles. Animals were fed a soy protein-free semipurified diet and had free access to daily renewed water.

In the first experiment, 25 rats were surgically ovariectomized (OVX; $n = 20$) or sham-operated (SH; $n = 5$). On the first day after surgery (day 0), OVX rats were randomized in four groups of five animals receiving either genistein (G; 10 μg/g BW/d), or daidzein (D; 10 μg/g BW/d), or 17α-ethinylestradiol (E; 23 μg/kg BW/d), or being untreated (OVX controls). As we previously demonstrated that feeding ovariectomized rats for three months with genistein or daidzein prevented the estrogen deficiency-induced bone loss while not exhibiting any uterotrophic effect [12], the genistein-, daidzein-, or 17α-ethinylestradiol-treatments (Sigma, France) were added into the diet over a 3-month-period. The daily diet quantity distributed to each ovariectomized rat was adjusted to the mean level consumed by SH, the previous day. Before sacrifice, vaginal smears were performed and stained using the Papanicolaou method (Papanicolaou reagents, Sigma, France). Then, on day 90, animals were killed by cervical dislocation, and uterine horns immediately collected, weighted, and used to test their contractile activity.

After this first long-term experiment in ovariectomized rats, a second experiment was carried out to study the acute effect of genistein or daidzein on the uterus of intact rats. For this purpose, four groups of five intact rats were s.c. injected with either genistein (G; 10 μg/g BW) or daidzein (D; 10 μg/g BW), previously dissolved in dimethylsulfoxide (DMSO) and diluted in sesame oil, or estradiol benzoate diluted in sesame oil (E; 23 μg/kg BW) (Intervet Laboratory, France), or the same amount of vehicle (C; control animals). 24 h after the injection, rats were killed by cervical dislocation, and uterine horns immediately collected to test their contractile activity.

2.1.2. In vitro uterine contractile activity

As previously described [1], the dissected uterine horns were quickly suspended in a tissue chamber (100 ml) filled with a Dejalon solution (pH 7.4) containing (mM) NaCl (155), KCl (5.7), $CaCl_2$ (0.55), $NaHCO_3$ (6.0) and glucose (2.8). The solution was equilibrated with 5% carbon dioxide and 95% oxygen, and kept at 32°C. A resting tension (1 g) was applied to the suspended uterine horns following addition to the tissue bath of OT and $PGF_2\alpha$ or its analogous compound, L, (Intervet Laboratory, France). In the first experiment, OT and $PGF_2\alpha$ were tested at $10^{-12}/10^{-11}$ M and $10^{-12}/10^{-9}$ M, respectively. In the second experiment, six increasing concentrations ranging from 10^{-15} to 10^{-10} M for OT and from 10^{-12} to 10^{-7} M for L were used. For each horn, the stimulation induced by each dose was evaluated as the sum of the basal level increases during the stimulation time and as the mean amplitude of contractions, which were converted into tension (as g) with a force–displacement transducer (Narco, F-60 model) and recorded on a stripchart recorder (Physiograph Narcosystems) (recorder speed: 2.5–3 cm per 10 min), as previously described [1]. Whatever the treatments and stimulations were, contraction frequency remained stable. In the first experiment, the uterine tension recorded for each dose (T) was shown. In the second experiment, the basal uterine tension (T_0) obtained without OT or L addition (0 M dose) was subtracted from T, and, for each contractile agent, a dose-response curve showing $T - T_0$ in terms of the substance concentration was determined.

2.1.3. Statistical methods

Results were expressed as means ± standard errors of means (SEM). All data were analyzed using the GraphPad InStat software (Microsoft, San Diego, CA, USA). Non-parametric methods were selected; a Kruskal-Wallis test was first performed; if it indicated a significant difference among groups ($P < 0.05$), the Mann-Whitney U test was used to determine specific differences.

2.2. Affinity of genistein and daidzein for the estrogen receptor

As previously described for rat uterus [13], radioligand binding assays were performed using calf uterus ER and [^{3}H] estradiol (1 nM). Following incubation (20 h; 4°C), the cytosol fractions were mixed with a dextran-coated charcoal suspension for 10 min at 4°C, and centrifuged. The bound radioactivity remaining in the collected charcoal supernatant was measured by liquid scintillation (scintillation counter: LS 6000, Beckman) (liquid scintillation cocktail: Formula 989, Packard). Parallel incubations were carried out in the presence of a 3000-fold excess of unlabelled 17β-estradiol, to determine non-specific binding. Then, specific radioligand binding to ER was defined as the difference between total binding and non-specific binding. To get competition curves showing the percentage of specific binding (mean value) in terms of the phytoestrogen concentration, genistein and daidzein, previously dissolved in DMSO, were tested at seven concentrations ranging from 0.1 nM to 100 μM in duplicate. A competition curve was also performed with the reference ligand (17β-estradiol) tested at eight concentrations in duplicate, for each experiment. Then, using non-linear regression analysis of competition curves and Hill equation curve fitting, the genistein or daidzein concentration inducing a half-maximal inhibition of specific binding (IC_{50}), and the Hill coefficient (nH) corresponding to the slope at the half-saturation point and indicating the binding co-operativity degree, were determined.

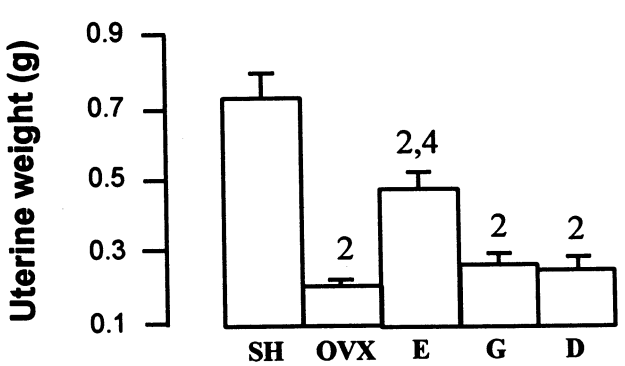

Fig. 1. Uterine weight in sham-operated (SH), ovariectomized (OVX), ovariectomized + 17α-ethinylestradiol (E), ovariectomized + genistein (G) and ovariectomized + daidzein (D) rats. Results are expressed as means ± SEM, for $n = 5$. (2) $P < 0.01$ vs. SH; (4) $P < 0.01$ vs. OVX.

3. Results

3.1. Uterine responsiveness to oxytocin and $PGF_2\alpha$ or luprostiol in phytoestrogen-treated rats

3.1.1. First experiment

Ovariectomy induced a decrease in uterine weight (g) (0.21 ± 0.02 vs. 0.73 ± 0.07 in SH; $P < 0.01$), which was prevented by the 17α-ethinylestradiol treatment (0.48 ± 0.05; $P < 0.01$ vs. OVX or SH). By contrast, uterine weight in genistein or daidzein-treated animals was not different from that measured in OVX (Fig. 1). In the same way, vaginal smears showed mainly leukocytes in OVX, G and D groups, while leukocytes and nucleated or even cornified epithelial cells were demonstrated in SH and E, respectively. Again, the basal uterine tension, which was lower in OVX, G, and D than in SH, was higher in E than in SH or OVX. Moreover, in phytoestrogen-treated animals, values were lower than in E (Fig. 2A and B). After OT addition, uterine tension in SH, but not in OVX, E or G, was increased compared to the basal tension. In daidzein-treated rats, it was only stimulated by the highest OT dose (Fig. 2A). Similar results were obtained after $PGF_2\alpha$ addition at 10^{-12} and 10^{-9} M (Fig. 2B).

3.1.2. Second experiment

At necropsy, the thin uterine horns in G and D groups differed from the fluid-full E ones, which were similar to the C ones. After OT addition, $T - T_0$ (g) progressively increased in the C group to reach a maximal level (2.15 ± 0.41) from the 10^{-11} M dose. Similar results were obtained from a smaller dose in the E group, as the maximal level (3.58 ± 0.72) was reached at 10^{-12} M. However, differences between the two maximal levels were not significant. By contrast, in G and D groups, whatever the OT dose was, we did not observe any significant increase in $T - T_0$, which was lower in G or D than in E with the 10^{-14} to 10^{-12} M doses, or in D than in E with the 10^{-11} and 10^{-10} M doses (Fig. 3A). After L addition, $T - T_0$ (g) significantly increased from the 10^{-10} M dose to reach a maximal level (5.91 ± 0.52) at 10^{-8} M in the C group. Similar results were demonstrated in the E group. By contrast, in G and D, $T - T_0$ significantly increased from the 10^{-8} M dose (Fig. 3B).

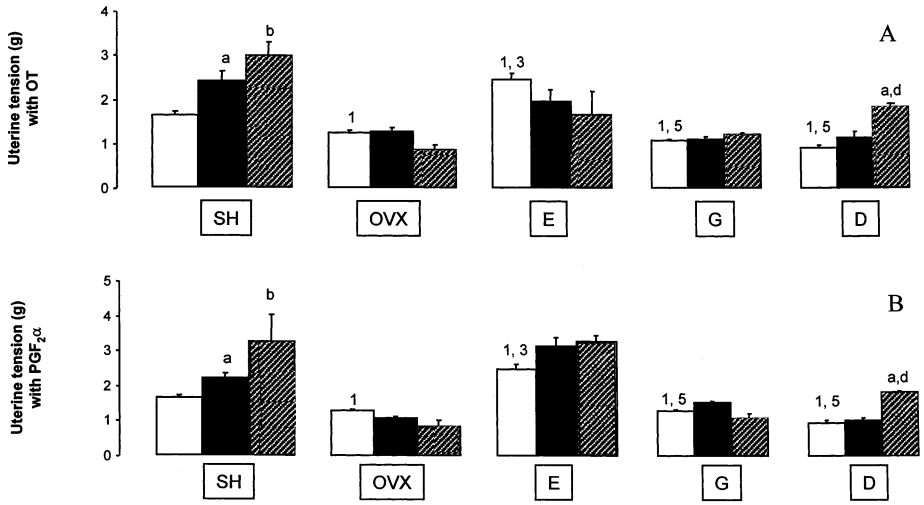

Fig. 2. In vitro uterine responsiveness to oxytocin (OT) (A) or prostaglandin $F_2\alpha$ ($PGF_2\alpha$) (B) in sham-operated (SH), ovariectomized (OVX), ovariectomized + 17α-ethinylestradiol (E), ovariectomized + genistein (G) and ovariectomized + daidzein (D) rats. White bars: 0 M, corresponding to the basal uterine tension. Black bars: 10^{-12} M. Gray bars: 10^{-11} M. Hatched bars: 10^{-9} M. Results are expressed as means ± SEM, for $n = 5$. (a) $P < 0.05$ vs. 0 M; (b) $P < 0.01$ vs. 0 M; (d) $P < 0.05$ vs. 10^{-12} M; (1) $P < 0.05$ vs. SH; (3) $P < 0.05$ vs. OVX; (5) $P < 0.05$ vs. E.

3.2. Affinity of genistein and daidzein for the estrogen receptor

As shown by the IC_{50} values determined for genistein, daidzein, or reference ligand (17β-estradiol) (Table 1), and by the corresponding competition curves obtained with genistein or daidzein (Fig. 4A and B, respectively), phytoestrogens inhibited the [^{3}H] estradiol binding to the calf uterus ER. A Hill coefficient of 0.8 was obtained with genistein, daidzein, or 17β-estradiol.

4. Discussion

Various studies focused on estrogen-related compounds, the selective estrogen receptor modulators (SERMs), exhibiting benefits on cardiovascular system, bone, or postmenopausal symptoms, but antagonizing estrogen action in breast. However, considering uterus, both estrogenic and anti-estrogenic properties were demonstrated, depending on the used SERM. In the same way, recent investigations on phytoestrogens and more particularly on soybean isoflavones indicate that these naturally occurring molecules may affect health by exhibiting tissue selective effects. However, as SERMs, soybean isoflavone effects on reproductive tissues such as uterus remain unclear, since they demonstrate both estrogenic and anti-estrogenic properties, depending on experimental conditions. Therefore, and as uterine contractile activity is partly under estrogen regulation in rats [14], the present study investigated the effect of genistein, daidzein, and estradiol on in vitro uterine responsiveness to OT and $PGF_2\alpha$ or L in ovariectomized or intact rats.

In our experimental conditions, genistein and daidzein did not exhibit any effect on ovariectomy-induced uterine atrophy, as previously shown [9,15]. By

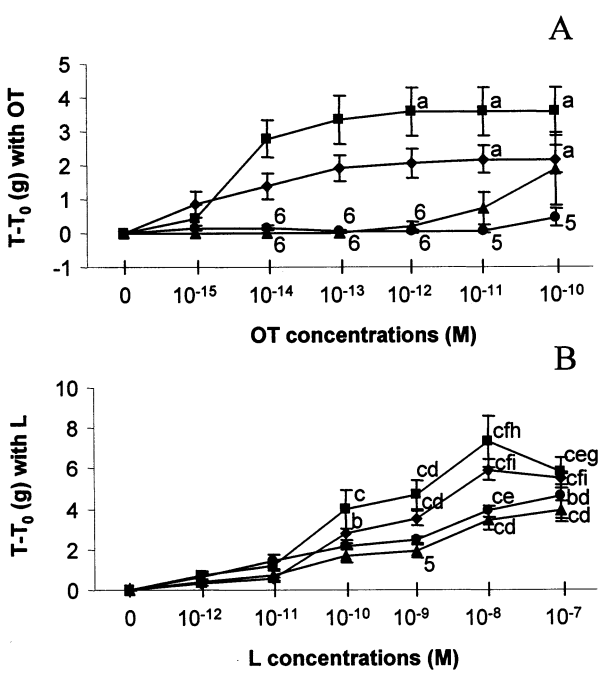

Fig. 3. In vitro uterine responsiveness to oxytocin (OT) (A) and luprostiol (L) (B) in control (◆), estradiol benzoate- (■), genistein- (▲) and daidzein-treated (●) intact rats. $T - T_0$ is the uterine tension registered for each substance concentration minus the basal uterine tension. Results are expressed as means ± SEM, for $n = 5$. (a) $P < 0.05$ vs. 0 M; (b) $P < 0.01$ vs. 0 M; (c) $P < 0.001$ vs. 0 M; (d) $P < 0.05$ vs. 10^{-12} M; (e) $P < 0.01$ vs. 10^{-12} M; (f) $P < 0.001$ vs. 10^{-12} M; (g) $P < 0.05$ vs. 10^{-11} M; (h) $P < 0.01$ vs. 10^{-11} M; (i) $P < 0.001$ vs. 10^{-11} M; (5) $P < 0.05$ vs. E; (6) $P < 0.01$ vs. E.

Table 1
17α-ethinylestradiol, genistein and daidzein concentrations inducing a half-maximal inhibition of the specific binding (IC_{50}) of [^{3}H] estradiol to the calf uterus estrogen receptor

	IC_{50} (nM)
17β-estradiol	1.7
Genistein	161
Daidzein	6650

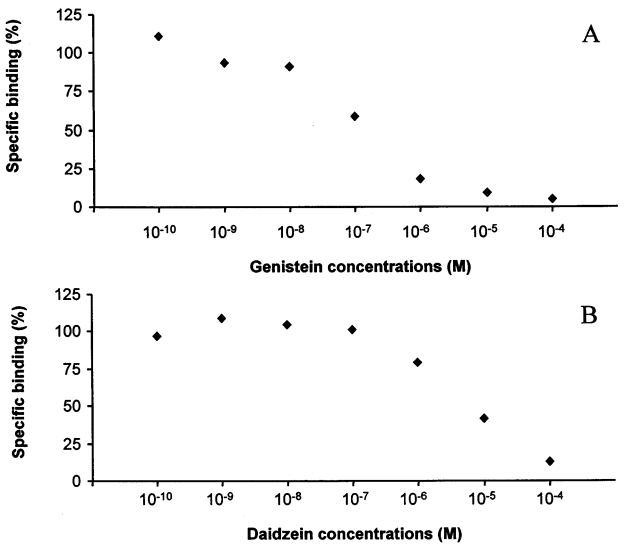

Fig. 4. Influence of genistein (A) or daidzein (B) on [^{3}H] estradiol binding to the calf uterus estrogen receptor (ER). Results are expressed as percentage of the specific binding of [^{3}H] estradiol to the ER.

contrast, daidzin but not genistin, both administered at the same dose and metabolized to daidzein and genistein, respectively, by gut bacteria glycosidases, was reported to prevent uterine atrophy in a dose-dependent manner [10]. In the same way, s.c. injected genistein was reported inefficient at 1 or 5 μg/g BW/d, while a 25 μg/g BW/d dose was associated with a trend towards a higher uterine mass [11]. Besides, equol which is naturally obtained by the gut microbial transformation of daidzein in ruminants, rats and humans [16,17] is known to be at least 10–100 and 10-fold more estrogenic than daidzein and genistein, respectively, in Siberian sturgeon [18]. Thus, we might suspect an equol action in daidzein-treated rats. Indeed, equol was detected in plasma of ovariectomized rats receiving daidzein at 10 μg/g BW/d per os for three months [12]. However, in the present experiment, genistein and daidzein (or equol) did not exhibit any estrogenic effect as only 17α-ethinylestradiol induced an uterotrophic activity (Fig. 1). In the same way and in accordance with previous results [9], genistein and daidzein did not elicit any estrogenic effect on vaginal cytology, as an artificial metestrous/diestrous state was observed in G, D and OVX groups, while SH and E vaginal epithelium showed a diestrous end/proestrous beginning and an artificial proestrous, respectively. Considering now uterine contractile activity, whatever the contractile dose was, the stimulatory effect depended on a previous sensitization by estrogens, as shown in SH and OVX. However, in agreement with previous results [19], OT and $PGF_2\alpha$ did not induce any variation in tension in the E group (Fig. 2A and B): the myometrium might not be able to contract anymore after a maximal contraction level was previously reached. Concerning genistein and daidzein, they did not exhibit any estrogenic activity as shown by the basal uterine tension. However, daidzein but not genistein sensitized myometrial smooth muscle cells to the highest dose of contractile agents (Fig. 2A and B). As equol could be more estrogenic than genistein and daidzein, we might suspect an equol rather than a daidzein action.

With endogenous estrogens (about 35 to 50 pg/ml of plasma at the diestrous end/proestrous beginning in C [20]), the positive $T - T_0$ value in C indicates that OT (Fig. 3A) or L (Fig. 3B) stimulated myometrial smooth muscle cells, increasing uterine tension in a dose-dependent manner. Moreover, a saturation phenomenon occurred with OT at 10^{-11} M and L at 10^{-8} M, as uterine contractile activity did not increase anymore. Estradiol benzoate injection might contribute to raise plasma estrogen levels in E. As a result, although it was not significant, uterine contractile activity was higher than in C (Fig. 3A and B), and the maximal level beyond which uterine myometrium could not contract anymore was reached with a lower OT dose than in C. On the opposite, phytoestrogens did not exhibit any estrogenic effect as differences with E were observed (Fig. 3A). Furthermore, although it was not significant, uterine contractile activity appeared to be lower than in C (Fig. 3A and B), suggesting an anti-estrogenic rather than an estrogenic activity of phytoestrogens, daidzein being more efficient than genistein in decreasing uterine responsiveness to OT. However, as shown by IC_{50} values, a higher daidzein than genistein level is required to induce a half-maximal inhibition of the specific binding of [^{3}H] estradiol to the calf uterus ER (Table 1), indicating a greater competition with genistein. Thus, and as equol was reported to compete the estradiol binding to the rat uterus ER [21], we might consider an equol rather than a daidzein effect in daidzein-treated rats. Finally, the higher stimulation of uterine contractile activity with L at 10^{-8} and 10^{-7} M compared to the 10^{-12} to 10^{-9} M doses (Fig. 3B) seems to indicate that, when endogenous estrogens should be antagonized, only high contractile agent doses could stimulate uterine myometrium.

In human tissue cultures, 17β-estradiol induces proliferation of both uterine endometrial and myometrial cells [22]. The present study demonstrated that rat

myometrium responsiveness to contractile agents was related to previous sensitization by estradiol. In this way, myometrium contractions after OT, $PGF_2\alpha$ or L additions are stimulated in a dose-dependent manner until a maximal level, beyond which they cannot increase anymore. In the same way, when the maximal contraction level is previously reached after estradiol treatment, contractile agents are inefficient. This might be explained by the down-regulations relative to the action mechanisms of OT, $PGF_2\alpha$ and estradiol on uterus. In myometrial smooth muscle cells, estradiol regulates the membrane potential and the intracellular calcium concentration by acting on potassium channel density [23] and calcium channel density or affinity, and by inducing calcium channels. In addition, estradiol increases the density of supportive, contractile and communicative proteins [24]. It also sensitizes uterus to contractile agents by increasing uterine OT mRNA [25] and myometrial OT receptor concentration [26], and by stimulating $PGF_2\alpha$ production by endometrial glandular cells. Finally, uterine $PGF_2\alpha$ release is stimulated by OT [27], and $PGF_2\alpha$ increases OT receptor density [28] while prolonged exposure to OT down-regulates these receptors [29].

In ovariectomized rats receiving genistein or daidzein (10 μg/g BW/d for three months per os), plasma genistein, daidzein and equol concentrations were about 13 000, 6000 and 6000-fold greater, respectively, [12] than basal plasma estradiol levels usually measured in intact rats (7–17 pg/ml) [20]. Thus, in the present study, phytoestrogens might have bound to the rat uterus ER, inducing either a very weak estrogenic activity in ovariectomized animals or an anti-estrogenic effect in intact rats by competing the endogenous estrogen binding to the ER. Classically, after binding to ligand, the receptor exhibits a conformational change in its ligand binding domain [30], inducing displacement of heat-shock-proteins, dimerization of the receptor and interaction with comodulators; the complex can then bind to the DNA-ERE and the transcription complex to activate or repress the transcription activity. As with SERMs, a distinct conformational change in the receptor ligand binding domain might be attributed to phytoestrogens. Furthermore, their binding to the receptor also could involve new ERE, and/or variations in the interaction with comodulators. Additionally, a new ER subtype (ER_β) was recently discovered in rat [31] and in human [32]. In rat, the ER_β protein was found to be highly homologous to the classical ER_α protein previously cloned in the rat uterus [33], since there are 95.5% amino acid identity in the DNA binding domain and 53.5% homology in the C-terminal ligand binding domain [31]. However, it was demonstrated that, in ER_α-mutant mice, the residual binding of estrogens (about 5% of the ER_α level) was insufficient to induce an estrogenic response [34]. Moreover, cells expressing preferentially ER_β are more likely to respond to phytoestrogens, while tissues relatively more rich in ER_α, such as reproductive tissues, might be differently affected by phytoestrogens [7]. Changes in ER_α/ER_β ratio might influence the receptor dimerization, as both subtypes form homodimers (ER_α–ER_α)(ER_β–ER_β) or heterodimers (ER_α–ER_β), which bind to DNA with an affinity similar to that of ER_α and greater than that of ER_β homodimers [35]. As a result, the predominance of one of the three forms might explain differences in the target tissue response to estrogens, phytoestrogens, or SERMs. Furthermore, alternative EREs might also play a role in the response specificity: ER_α and ER_β exhibit similar effect on target genes under classical ERE control, but different effect on target genes under AP-1 site control [36]; only the estradiol/ER_α complex activates such a site, while tamoxifen (bound to ER_α or ER_β) stimulates it. In the rat uterus, both ER_α (66 kDa) and ER_β (55 kDa) proteins were detected: ER_α is present both in glandular and luminal epithelial cells, whereas ER_β is only present in glandular epithelial cells [37]. Moreover, before ovariectomy (inducing an ER_β mRNA suppression while maintaining the ER_α level), ER_α mRNA is 47-fold more represented than ER_β mRNA [38]. This change in ER_α and ER_β expression after ovariectomy might suggest that, in our first experiment, ER_α rather than ER_β was involved in the very weak estrogenic effect of phytoestrogens. Finally, in cells expressing the ER, as endogenous estrogens, phytoestrogens also may act through non-genomic effects mediated by membrane-bound ER's or other cellular proteins [7], inducing changes in cytoplasm second messengers and cellular activity impairments. In this way, genistein might inhibit phosphorylation of cytoplasm proteins via its anti-tyrosine kinase properties [39] and, as a result, alter cellular activities. In pregnant rat myometrial cells, genistein but not daidzein inhibits a calcium current via its tyrosine-kinase inhibitory properties, suggesting that modulation of these calcium channels by endogenous tyrosine-kinases may play a role on the regulation of calcium influx and uterine contractions during normal and preterm labors [40].

In conclusion, various action mechanisms might explain the phytoestrogen effects on in vitro rat uterine contractile activity. It seems possible that both genistein and daidzein (or equol) might bind to the ER, inducing anti-estrogenic effects on uterine responsiveness to contractile agents in intact rats. On the opposite, in conditions such as ovariectomy or with high concentrations of contractile agents, phytoestrogens might exhibit very weak estrogen-like effects on rat uterine contractile activity.

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