Nitric oxide sensitive-guanylyl cyclase subunit expression changes during estrous cycle in anterior pituitary glands

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Cabilla JP, Ronchetti SA, Nudler SI, Miler EA, Quinteros FA, Duvilanski BH. Nitric oxide sensitive-guanylyl cyclase subunit expression changes during estrous cycle in anterior pituitary glands. Am J Physiol Endocrinol Metab 296: E731–E737, 2009. First published January 13, 2009; doi:10.1152/ajpendo.90795.2008.—17β-Estradiol (E2) exerts inhibitory actions on the nitric oxide pathway in rat adult pituitary glands. Previously, we reported that in vivo E2 acute treatment had opposite effects on soluble guanylyl cyclase (sGC) subunits, increasing α₁- and decreasing β₁-subunit protein and mRNA expression and decreasing sGC activity in immature rats. Here we studied the E2 effect on sGC protein and mRNA expression in anterior pituitary gland from adult female rats to address whether the maturation of the hypothalamus-pituitary axis influences its effects and to corroborate whether these effects occur in physiological conditions such as during estrous cycle. E2 administration causes the same effect on sGC as seen in immature rats, and these effects are estrogen receptor dependent. These results suggest that E2 is the main effector of these changes. Since the sGC α-subunit increases while the sGC activity decreases, we studied if other less active isoforms of the sGC α-subunit are expressed. Here we show for the first time that sGCα2 and sGCα2 inhibitory (α2i) isoforms are expressed in this gland, but only sGCα2; mRNA increased after E2 acute treatment. Finally, to test whether E2 effects take place under a physiological condition, sGC subunit expression was monitored over estrous cycle. sGCα, β₁, and β₂i fluctuate along estrous cycle, and these changes are directly related with E2 level fluctuations rather than to NO level variations. These findings show that E2 physiologically regulates sGC expression and highlight a novel mechanism by which E2 downregulates sGC activity in rat anterior pituitary gland.

The main estrogenic hormone 17β-estradiol (E2) plays important regulatory roles in a broad variety of biological processes, acting mainly on reproductive tissues, bone, liver, pituitary, and brain (9, 25).

Nitric oxide (NO) is a signaling molecule that freely diffuses across cellular membranes where it binds to its main intracellular receptor, soluble guanylyl cyclase (sGC). This enzyme catalyzes the formation of cGMP from GTP. Subsequently, targets of cGMP such as cGMP-dependent protein kinases, cyclic nucleotide phosphodiesterases, and cyclic nucleotide-sensitive ion channels are activated to continue the signal transduction (15, 18).

sGC is an heterodimeric enzyme and is comprised of two subunits, α and β, of which four types exist (α₁, α₂, β₁, and β₂). Both α-isofoms form a functional enzyme with the β₁-subunit, although the α₁/β₁ is the most abundant and widely expressed heterodimer, showing the greater activity (12, 13). The α₂ is expressed in a more restricted pattern: in human tissues, it is present mainly in spleen, placenta, brain, and uterus; in rat, it was found in fetal brain (3). Furthermore, an inhibitory α₂-subunit (α2i) and many splicing variants of both isoforms of variable activity have also been identified (1, 22).

Reports indicate that E2 regulates the NO/sGC/cGMP pathway and the levels of NO and cGMP in many tissues. Several studies (21, 24) sustain an inhibitory role of E2 on the NO pathway in pituitary gland. Pituitary glands from ovariectomized rats show increased NO synthase activity and mRNA and protein levels, whereas E2 treatment reverts this condition. These E2 effects were only observed after in vivo treatment, and it has been suggested that they are indirect. In addition, it has been reported that E2 affects sGC expression and activity in uterus, PC12 cells, and hypothalamus (8, 10, 14). Previous studies from our laboratory (4) show that acute E2 treatment exerts an inhibitory effect on sGC by downregulating the sGCβ₁ subunit and sGC activity but increases sGCα1 expression in anterior pituitary gland from immature rats. E2 effects on anterior pituitary sGC were observed such after in vivo as in vitro treatment, suggesting a direct effect of E2 on sGC regulation and a differential and independent regulation of both subunits. Previous evidence (4, 7) further sustains that under certain conditions α₁ and β₁ can be independently regulated.

Taking into account preceding results, the aim of this work was to investigate whether E2 affects sGC subunit expression and activity on pituitary gland from adult rats and to corroborate whether these effects occur in physiological conditions such as during estrous cycle. Besides, since the sGC α-subunit increases while sGC activity decreases, we studied the expression of other less active isoforms of the sGC α-subunit in the anterior pituitary gland and whether they are regulated by E2. To this end, we studied the in vivo effect of exogenous or endogenous E2 on sGC expression.

MATERIALS AND METHODS

Materials

The 7α,17β-19[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]stramine (10)-triene-3,17-diol (ICI 182,780) was purchased from Tocris Neuramin (Bristol, UK). Z-1-2-aminoethyl)-N-(2-ammonioethyl)amino)diacetyl-1-ium-1,2-diolate (DETHANONOate) was purchased from Cayman Chemical (Ann Arbor, MI). Leupeptin, pepstatin, and PMSF were from Sigma (St. Louis, MO).

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Animals and treatments

Adult female Wistar rats (180–200 g) were used. Animals were kept with controlled conditions of light (12:12-h light-dark cycle) and temperature (21–24°C). Food and water were supplied ad libitum. All procedures were in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

For E2 acute effects experiments, rats were injected subcutaneously in the perisacral region with 40 μg/kg body wt E2 or with vehicle alone (propylene glycol) and killed over a time course. When required, animals were injected intraperitoneally with 2 mg/kg ICI 182,780 30 min before 40 μg/kg E2 administration.

Intact rats were monitored by daily (0800-0900) vaginal smears over three consecutive cycles. Animals at random stages of estrous cycle or at proestrus, estrus, or diestrus were killed by decapitation at 1700.

Ovariectomy

Rats were ovariectomized under ketamine (75 mg/kg; Holliday-Scott, Buenos Aires, Argentina) and xylazine (10 mg/kg; König, Buenos Aires, Argentina) anesthesia 14 days before the experiments. Sham-operated rats were used as controls.

Cell culture

Primary cell culture was prepared from anterior pituitary glands of ovariectomized or sham rats killed at 14 days postsurgery. Anterior pituitary glands from each condition were pooled for each cell culture. Cells were obtained by enzymatic (trypsin/DNAse) and mechanical dispersion (extrusion through a Pasteur pipette). Cell viability was assessed by the trypan blue exclusion method. Dispersed cells were seeded onto tissue culture plates and stabilized for 48 h (37°C, 5% CO2 in air) in phenol red-free DMEM supplemented with 10% charcoal stripped FBS, 10 μl/ml MEM amino acids, 2 mM glutamine, 5.6 μg/ml amphotericin B, and 25 μg/ml gentamicin.

Cell treatment

After the stabilization period, the medium was changed for fresh medium and cells were incubated during 6 h (37°C, 5% CO2 in air) with or without 0.5 mM DETANONOate. After treatment, RNA isolation from each condition was carried out.

Immunoblot analysis

Anterior pituitary glands were removed and sonicated in lysis buffer containing 10 mM HEPES pH 7.4, 150 mM NaCl, 10 mM EDTA, 100 μM leupeptin, 350 μM pepstatin, 0.5 mM PMSF, and 0.2 mM DTT. Sonicates were centrifuged for 20 min at 10,000 g and the soluble fraction was used in the immunoblot analysis. Protein content from the supernatants was measured by Bradford reagent, using BSA as a standard. Twenty to thirty micrograms of total protein from each sample were boiled for 5 min in Laemmli sample buffer and were fractioned on 10% SDS-PAGE. Resolved proteins were transferred to polyvinylidene difluoride membranes and blocked for 2 d at 4°C in blocking buffer (TBS-0.05% Tween 20 and 6% nonfat dry milk). Then, membranes were incubated overnight at 4°C with rabbit antisera anti-GCα1 (1:1,750) or β1 (1:700) subunits and anti-actin (1:1,000) in blocking buffer. Then blots were washed and incubated for 1 h at room temperature with horseradish-peroxidase conjugated goat anti-rabbit IgG (1:2,000), followed by detection of immunoreactivity with diaminobenzidine solution containing 0.01% hydrogen peroxide.

RT-PCR and semiquantitative PCR

RNA isolation. Tissues were removed and immediately homogenized with TRIzol reagent. After isolation, total RNA from tissues was spectrophotometrically quantified at 260 nm. RNA integrity was checked in formaldehyde/formamide gel electrophoresis.

RT and PCR reactions. First-strand cDNA was synthesized with Moloney murine leukemia virus RT in RT buffer containing 5.5 mM MgCl2, 0.5 mM dNTP, 2.5 μM random hexamers, and 3.125 U/μl Moloney murine leukemia virus RT. Reactions were done in a final volume of 12 μl containing 1 μg RNA. The RT reaction was run at 37°C for 50 min, and RT was inactivated by heating the samples at 70°C for 15 min before the PCR reactions. To check for genomic contamination, the same procedure was performed on samples in a reaction solution lacking RT.

Specific primers for both subunits of sGC were designed from published sequences (23) with Oligo Perfect designer software (Invitrogen) and are detailed in Table 1. The amplified products spanned from nucleotide position base 1,971 to 2,054 in the C-terminal region of sGCα1, from 714–823 in the N-terminal region of sGCβ1, from 1,530–1,929 in the C-terminal region of sGCα2, and from within the in-frame insert to 275 bp to the 3’-end of sGCα2 (1). β-Actin was used as an endogenous control. Actin primers were designed to detect amplification of DNA contamination. Then, samples were thermocycled for PCR amplification (Mastercycler; Eppendorf, Hamburg, Germany). The reaction mixture contained GoTaq PCR buffer, 1.5 mM MgCl2, 200 μM of each dNTP, 0.625 U GoTaq polymerase, and 300 nM of each primer. RT-PCR methods were utilized to determine relative changes in mRNA expression. Reactions were subjected to a varying number (n = 16–40) of cycles of PCR amplification (melting phase 94°C for 30 s, annealing 55°C for 30 s, and extension 72°C for 1 min) to find out the optimum cycle number within the linear range for PCR amplification. Amplified products collected at various cycles were analyzed by electrophoresis in 1.5% agarose-ethidium bromide gels, and the optimum cycle number resulted in 24 cycles for sGCα1, 28 cycles for sGCβ1, and 40 cycles for sGCα2 and sGCα2.

Analysis of semiquantitative PCR and immunoblot data

The intensities of PCR products and immunoblot signals were determined by digital image analysis using the Gel Pro analyzer

Table 1. Primers used for semiquantitative RT-PCR assays

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Product Size</th>
</tr>
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<tbody>
<tr>
<td>sGCα1</td>
<td>Forward</td>
<td>83 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td></td>
</tr>
<tr>
<td>sGCβ1</td>
<td>Forward</td>
<td>109 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td></td>
</tr>
<tr>
<td>sGCα2</td>
<td>Forward</td>
<td>399 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>Forward</td>
<td>275 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td></td>
</tr>
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sGC, soluble guanylyl cyclase; i, inhibitory isof orm.
E2h :9 8

sured at different times. sGC activity was significantly reduced to an increase in sGCα1 and sGCβ1 mRNA expression by semiquantitative PCR. At 4- and 8-h post-E2 injection, sGCα1 mRNA was significantly augmented respect to control values (Fig. 1). At the same time points, sGCβ1 mRNA levels were diminished, consistent with the observed at protein level. These findings indicate that the E2 effects on sGC expression are mediated by ER activation.

E2 acute treatment increases the expression of the inhibitory subunit α2i.

It is known that other isoforms of sGCα are expressed in different tissues. To date, the presence of these isoforms was not reported in pituitary gland. In our case, the augment registered in sGCα1 protein as well as in mRNA could be due to an increase in α1 and/or to other less active or inhibitory isoforms of sGCα. In the present work, the anti-sGCα1 antibody utilized for immunoblot studies, as well as the primers used to amplify sGCα1 mRNA, was directed to the C-terminal sequence of rat sGCα1 and it cannot differentiate among sGCα species, since all of them include this sequence. To examine if these subunits are expressed in this tissue, we performed RT-PCR to detect sGCα2 and α2i, using specific primers and liver and kidney or spleen as control tissues, respectively. Here we show for the first time that sGCα2 and α2i are expressed in anterior pituitary gland (Fig. 3A). Then, to investigate if their expression was modified by E2 treatment, rats were treated with a single dose of E2 and killed after 6 h. E2 treatment did not modify α2 mRNA expression respect to control, but α2i mRNA levels were dramatically increased (relative units as percentage of control; sGCα2 control: 100 ± 24; E2 8 h: 94 ± 30; sGCβ2 control: 100 ± 18; and E2 8 h: 1,730 ± 105; \( P < 0.001\), Dunnett’s test; \( n = 3\); Fig. 3B). Again, to verify if this effect was E2 specific, rats were injected intraperitoneally with 2 mg/kg IC 182,780 30 min before E2 subcutaneous administration, and killed over 8 h. The inhibitor was able to fully...
abolist \( \alpha_2 \) expression increase and had no effect per se on sGC\( \alpha_2 \) (Fig. 3C). Thus the augmented sGC\( \alpha \) expression may reflect \( \alpha_1 \)-increased levels but also an augmented \( \alpha_2 \) expression. These findings suggest that both \( \beta_1 \) downregulation and \( \alpha_2 \)-augmented expression could contribute to the acute inhibitory effect of E2 on sGC.

**sGC subunit expression levels are variable through estrous cycle**

Taking into account the acute E2 actions on sGC expression and activity and to examine whether these effects occur under physiological conditions, we studied the changes on sGC through estrous cycle, a condition where E2 and also gonadotrophins, prolactin, and other gonadal steroids undergo rapid, dramatic changes. To this end, rats were killed on the afternoon (1700) of each stage of the estrous cycle and sGC protein levels were analyzed by Western blot. Protein levels of sGC\( \alpha_1 \) showed a significant increase through the cycle from proestrus to estrus but significantly decreased at diestrus. Similar results were observed when sGC\( \alpha_1 \) and \( \beta_1 \) mRNA expression was evaluated by RT-PCR (data not shown). These findings show that sGC subunits levels independently fluctuate in vivo during estrous cycle. These individual variations of sGC subunit expression are consequence of hormonal changes taking place during estrous cycle, because in male rats and in ovariectomized rats both subunits show similar mRNA levels (relative units expressed as means \( \pm \) SE; \( n = 3 \); male sGC\( \alpha_1 \): 1.41 \( \pm \) 0.15 and sGC\( \beta_1 \): 1.54 \( \pm \) 0.2; and 14-day ovariectomized sGC\( \alpha_1 \): 1.43 \( \pm \) 0.42 and sGC\( \beta_1 \): 1.61 \( \pm \) 0.23).

**sGC\( \alpha_2 \) expression levels are variable through the estrous cycle**

Bearing in mind our findings showing that sGC\( \alpha_1 \) expression increases over the cycle in spite of the decrease in cGMP production, the sGC\( \alpha_2 \) contribution to global expression was addressed. In accordance with the enhanced sGC activity on proestrus, \( \alpha_2 \) mRNA expression was the lowest at this stage (Fig. 4B). On estrus and diestrus, \( \alpha_2 \) expression was significantly augmented, which correlates with a lesser cGMP production. Altogether, the difference between sGC subunit expression according to the stage of the estrous cycle in which the animals were killed suggests the cyclicity of the response.

**Role of NO on sGC subunit expression**

NOS I protein and activity are strikingly upregulated on the afternoon of proestrus, and both return to basal levels after the afternoon of estrus, remaining low on diestrus I and II. cGMP production strongly correlates with NOS I upregulation (17). To determine if the changes seen in sGC subunit protein levels were due to changes in NO levels, we studied the in vitro effect of a short-time NO and E2 exposition on sGC protein expression. Pituitary cell cultures from intact adult female rats were incubated with 0.1 mM DETANONOate, a NO donor, or \( 10^{-9} \) M E2 for 6 h, and sGC\( \alpha_1 \) and \( \beta_1 \) expression was evaluated by Western blot. NO treatment upregulated both subunits in a

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*Figure 2. E2 actions on sGC protein expression are abolished by pure anti-oestrogenic ICI 182,780 pretreatment in rat anterior pituitary gland. Adult intact rats at random stages of estrous cycle (\( n = 5 \) per group) were injected subcutaneous with 2 mg/kg ICI 182,780 (ICI) 30 min before 40 mg/kg body wt E2 injection and killed after 8 h. Top: representative Western blot. Bottom: average densitometric values. Bars represent media \( \pm SE \) of the densitometric values of sGC\( \alpha_1 \) (open bars) and sGC\( \beta_1 \) (solid bars) protein levels normalized to actin, as percentage of control (\( n = 3 \)). *\( P < 0.05 \), **\( P < 0.01 \) vs. respective controls; \( \Delta \)\( P < 0.05 \), \( \Delta \Delta \)\( P < 0.01 \) vs. E2, ANOVA followed by Dunnett’s test.*

*Figure 3. Other isoforms of sGCs are expressed normally in rat anterior pituitary gland. A: representative PCR showing the presence of sGC\( \alpha_1 \) and \( \alpha_2 \), mRNAs in anterior pituitary. Liver, kidney, and spleen were used as respective control tissues. B: acute E2 exposition increases the mRNA expression of the dominant negative isoform sGC\( \alpha_2 \). Animals were injected with 40 mg/kg body wt E2 and killed after 8 h. C: role of ER in sGC\( \alpha_2 \)-augmented expression. Adult intact rats at random stages of estrous cycle (\( n = 3 \) per group) were injected intraperitoneally with 2 mg/kg ICI 182,780 30 min before 40 mg/kg body wt E2 injection and killed after 8 h. Bars are means \( \pm SE \) of the densitometric values of sGC\( \alpha_1 \) mRNA normalized to actin, as percentage of control (\( n = 3 \)). *\( P < 0.05 \) vs control; \( \Delta \)\( P < 0.05 \) vs. E2, ANOVA followed by Tukey’s test.*
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Tukey’s test.

Fig. 4. sGC subunit expression fluctuates over estrous cycle. Rats (n = 3 per group) were killed at 1700 on proestrus, estrus, or diestrus. A, top: a representative Western blot; bottom: average densitometric values. Bars are means ± SE of sGCα1 (open bars) and sGCβ1 (solid bars) protein values normalized to actin (n = 5). *P < 0.05 vs. estrus, ANOVA followed by Tukey’s test. B, top: a representative PCR of sGCα2i; bottom: average densitometric values. Bars are means ± SE of sGCα2i mRNA levels normalized to actin (n = 3). *P < 0.05 vs. estrus and diestrus, ANOVA followed by Tukey’s test.

Fig. 5. Short-time nitric oxide exposure upregulated sGC expression, while E2 acute expression differentially affects sGC subunits in vitro. A: primary pituitary cell cultures from intact adult rats at random stages of estrous cycle were incubated with 0.5 mM DETANONOate, a nitric oxide donor, or with culture medium alone (control) for 6 h. Top: representative Western blot. Bottom: average densitometric values. Bars are means ± SE of sGCα1 (open bars) or sGCβ1 (solid bars) protein levels normalized to actin, as percentage of control (n = 5). B: primary pituitary cell cultures from intact, adult rats were incubated with 10−9 M E2 or with culture medium alone (control) for 6 h. Top: representative Western blot. Bottom: average densitometric values. Bars are means ± SE of sGCα1 (open bars) or sGCβ1 (solid bars) protein levels normalized to actin, as percentage of control (n = 3). *P < 0.05 vs. respective control, Student’s t-test.

Discussion

Here we have shown that E2 causes the same effect on sGC subunits in adult and juvenile female animals, suggesting that these effects are distinctive of E2 and independent of the hormonal fluctuation during the maturation of the reproductive axis. In vivo E2 acute treatment exerted opposite actions on both sGC subunits, increasing sGCα1 mRNA and protein levels and, simultaneously, decreasing sGCβ1 mRNA and protein levels. These effects were E2 specific and ER dependent. Our results also demonstrate that α- and β-subunit levels are independently modified. Different authors have previously reported that under certain conditions α- and β-subunits can be individually regulated. A complete loss of the β1-subunit in aortic smooth muscle cells from old rats has been found, while the α-subunit is still present (7). In developing rat brain, only sGCα mRNA is expressed, while sGCβ1 is totally absent (26), raising interesting questions regarding the independent functions of each subunit.

We have demonstrated that E2 acute treatment enhanced α1 mRNA and protein expression but decreased sGC activity. It is known that fully active sGC requires α- and β-subunits in a strict 1:1 stoichiometry, and, even considering that α1 expression was increased, sGCβ1 downregulation would be enough to reduce sGC global activity. However, the higher expression of α1 mRNA could be due, at least in part, to an increased expression of other α-isoisomers with less activity. In the present work, we have demonstrated for the first time that sGCα2 and sGCα2i isoforms are expressed in anterior pituitary gland and that after E2 stimulus sGCα2 expression (but not α2) is augmented. The similar fashion (Fig. 5A) while E2 treatment increased α1 but decreased β1 expression (Fig. 5B). In addition, sGCα1 and β1 expression was studied in the anterior pituitary glands of 14-day ovariectomized rats when NOS I protein and activity were markedly upregulated. At this condition, the results were similar to those with the NO donor in vitro (relative units as percentage of control; sGCα1: 120.7 ± 7.5 and sGCβ1: 126 ± 10.7; P < 0.05, ANOVA followed by Dunnett’s test; n = 3). Thus short-time in vitro NO treatment or chronic in vivo NO increase did not mimic the effect of E2 in vitro or those seen in vivo during estrous cycle on sGC subunits. These results suggest again that the imbalance of sGC subunit expression is likely due to hormonal fluctuations rather than to NO.
fact that the inhibitory α isoform could collaborate to E2 transient sGC inhibition indicates again that E2 is acting through multiple pathways. Therefore, our results show that E2 not only decreases sGCβ1 expression but also stimulates sGCα2 expression and by these ways participates in sGC activity downregulation.

The estrous cycle is a physiological event in which mainly E2 levels, among other hormones, suffer strong changes (27). We studied the expression of both sGC subunits during the estrous cycle to address whether the changes in E2 levels are reflected in the expression pattern of sGC in anterior pituitary gland. Results from this work show that sGCα- and β-subunits levels fluctuate through estrous cycle, further supporting a correlation between E2 level changes and sGC expression pattern. On diestrus, when E2 levels are rising and NO production is at baseline levels, the expression of sGCβ1 is the lowest of the entire cycle and, conversely, sGCα1 expression is maximal. Later, on the afternoon of proestrus, when serum E2 levels have drastically fallen and NO production is high, sGCα1 expression decreases, while a marked increase in sGCβ1 expression is observed. However, the changes in sGC subunit expression are not identical to that obtained after E2 acute administration; there is a shifting towards diestrus where the maximal expression of α1 was observed. It is possible that the slow but continuous increase in serum E2 levels, beginning on diestrus I, would be enough to cause the differential changes in the sGC subunits on diestrus.

Our results show that E2 treatment enhances sGCα expression, including an augment in α2i. However, the proportion of α2i vs. α1i expression is very low. Why does E2 augment sGCαi expression if it would not be involved in sGC enzyme constitution? There is some evidence that strongly suggests that the sGCα1 subunit can be individually involved in other processes, independently of cGMP production. In advanced prostate cancer, sGCα1 levels are highly correlated with proliferation (5). E2 as a mitogenic factor, as well as a proapoptotic stimulus, drives changes in pituitary cell population (11, 16, 20). sGCαi levels and timing of pituitary cell proliferation seems to be correlated. sGCα1 expression raises on estrus and diestrus, while proliferation events are taking place, and remains low on proestrus, when the highest levels of apoptotic cells are detected (6, 19, 28). Thus the putative role of α1 in anterior pituitary cell renewal opens a very attractive landscape that is now under investigation.

NO modifies sGC expression on various tissues (15), and NOS expression and activity in anterior pituitary are variable and susceptible of regulation during estrous cycle (17). Here we show that both short- and long-time NO exposition upregulated both sGC subunits at the same extent. Therefore, NO seems not to be involved in the differential changes of the sGC subunit expression during the estrous cycle.

In summary, we provide evidence that sGC activity is downregulated and its subunits are independently affected upon stimulus with exogenous E2 and during the estrous cycle in the rat anterior pituitary gland. These effects appear to be independent of the hypothalamic-pituitary-gonadal axis maturation. The expression of other less active or inhibitory sGC isoforms provides a new potential regulation point in sGC activity. The study of the roles of each subunit of sGC in other processes, in addition to the classical cGMP-producing function, will raise new perspectives and novel pathways to a well-known enzyme.

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