Sildenafil treatment in vivo stimulates Leydig cell steroidogenesis via the cAMP/cGMP signaling pathway

Silvana A. Andric,* Marija M. Janjic, Natasa J. Stojkov, and Tatjana S. Kostic*

Reproductive Endocrinology and Signaling Group, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

Submitted 7 June 2010; accepted in final form 25 July 2010

Andric SA, Janjic MM, Stojkov NJ, Kostic TS. Sildenafil treatment in vivo stimulates Leydig cell steroidogenesis via the cAMP/cGMP signaling pathway. Am J Physiol Endocrinol Metab 299:E544–E550, 2010. First published July 27, 2010; doi:10.1152/ajpendo.00337.2010.—Sildenafil citrate (Viagra), a cGMP-selective phosphodiesterase (PDE) inhibitor, is widely used to treat erectile dysfunction and pulmonary arterial hypertension. In contrast to its well established action on erectile dysfunction, little is known on the action of sildenafil on cGMP/cAMP signaling and testicular steroidogenesis. This study was designed to assess the effects of prolonged sildenafil treatment on NO synthase-dependent signaling and steroidogenic function of rat Leydig cells. Male adult rats were treated with Viagra (1.25 mg/kg body wt) daily for 30 days. In our studies, serum testosterone and ex vivo testosterone production significantly increased in sildenafil-treated animals. Human chorionic gonadotropin-stimulated testosterone production and cAMP accumulation were also significantly higher in Leydig cells obtained from sildenafil-treated rats. The expression of soluble guanylyl cyclase (GUCY1) subunits (Gucy1a, Gucy1b1) significantly increased; cAMP-specific Pde4a, cGMP-specific Pde6c, and dual Pde1c and Nos2 were inhibited and expression of Nos3, protein kinase G1 (Pkg1), and Pde5 remained unchanged. Treatment of purified Leydig cells with NO donor caused a dose-dependent increase in both testosterone and cAMP production. Testosterone and cGMP production was significantly higher in Leydig cells obtained from sildenafil-treated animals. The stimulatory effect of NO donor was significantly enhanced by saturating concentrations of hCG in both Leydig cells obtained from control and sildenafil-treated animals. Occurrence of mature steroidogenic acute regulatory protein also increased in sildenafil treated animals in accord with increased cAMP and cGMP production. In summary, inhibition of PDE activity during prolonged sildenafil treatment increased serum testosterone level and Leydig production. In summary, inhibition of PDE activity during prolonged sildenafil treatment increased serum testosterone level and Leydig production.

Steroidogenic acute regulatory protein; cyclic adenosine monophosphate; cyclic guanosine monophosphate

IN MALES, ANDROGENS ARE ESSENTIAL for puberty, fertility, sexual motivation, and sexual performance (16). Testosterone production is predominantly regulated through interaction of luteinizing hormone (LH)/human chorionic gonadotropin (hCG) with its specific receptor (6, 11), resulting in increased intracellular cAMP level and subsequent activation of cAMP-dependent protein kinase (PKA). PKA phosphorylates numerous proteins including those that facilitate cholesterol trans-

* S. A. Andric and T. S. Kostic contributed equally to this work.

Address for reprint requests and other correspondence: T. S. Kostic, Reproductive Endocrinology and Signaling Group, Dept. of Biology and Ecology, Faculty of Sciences at Univ. of Novi Sad, Dositeja Obradovica Sq. 2, 21000 Novi Sad, Serbia (e-mail: tatjana.kostic@dbe.uns.ac.rs).
MATERIALS AND METHODS

**Materials.** The antisera for StAR protein were generous gifts from Prof. Douglas Stocco (7) and Dale B. Hales (15), while the purified rabbit polyclonal antibody against PDE5 was a generous gift from Prof. Sergei Rybalkin (28). Commercial antibodies and all other reagents are listed in detail and given as supplemental material (Supplemental material can be found in the online version of this paper at the Journal website). Sildenafil (Viagra) in tablets was from Pfizer (www.Pfizer.com).

**Ethical approval.** All the experimental protocols were approved by the Ethics Committee on Animal Care and Use at the University of Novi Sad, operating under the rules of National Council for Animal Welfare and following statements of National Law for Animal Welfare (copyright March 2009). All our experiments were performed and conducted in accordance with the National Research Council (NRC) publication *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, Washington DC, 1996) and NIH *Guide for the Care and Use of Laboratory Animals* (NIH Publications, No. 80 23, revised, 7th ed., 1996). All the experiments were carried out in the Laboratory for Reproductive Endocrinology and Signaling, DBE, Faculty of Sciences at University of Novi Sad.

**Animals and treatment.** Adult (3 mo old, 250–270 g body wt) male Wistar rats, bred and raised in the Animal Facility of the Faculty of Sciences (University of Novi Sad), were used for the experiments. The animals were raised in controlled environmental conditions (22 ± 2°C, 12:12-h light-dark cycle, lights on at 0700) with food and water ad libitum. Animals were divided into two groups, each consisting of eight adult male rats: 1) Control group, received pure distilled water daily for 30 days and 2) sildenafil-treated group, received sildenafil (Viagra; www.Pfizer.com) dissolved in distilled water by oral dosing (1.25 mg/kg body wt) daily for 30 days. Experiments were repeated at least three times. At the end of the experimental period, both groups were quickly decapitated, serum was collected, serum samples were stored at −20°C until assaying for androgens (T+DHT) levels, and ex vivo experiments were performed.

The methodology for the studies included in this article is briefly outlined here, but it was previously reported by our group (1, 2, 19, 20) and is given with all details in the Supplemental material.

**Preparation of leydig cells and ex vivo androgens, NO, and cAMP/cGMP production.** To follow ex vivo steroidogenic function, we used Leydig cells obtained from control and sildenafil-treated rats. Primary cultures of purified Leydig cells were prepared from suspensions of interstitial cells, as previously described by our group (1, 2, 19, 20). The proportion of Leydig cells present in culture was determined by staining for HSD-3b activity (24) and was found to be 97.9 ± 1.4%, while the viability was more than 90%. (1, 2, 19, 20). Purified Leydig cells obtained individually from eight male rats were pooled and plated in Petri dishes (3 × 10^6 cells/well) were lysed as described previously (1, 2, 19, 20). Three to five replicates of each cell/group were cultured for ex vivo secretion and expression analysis. After 2 h, cell-free medium was collected and stored at −80°C prior to the measurement of androgens levels, while cAMP and cGMP were measured in cell content. For the expression analysis of transcripts/proteins, Leydig cell lysates were used as a source of RNA/protein.

**Androgens, NO, and cAMP/cGMP measurement.** Androgens levels were measured by radioimmunoassay (1, 2, 19, 20). Levels of androgens are referred to as testosterone + dihydrotestosterone (T+DHT) because the anti-testosterone serum no. 250 showed 100% cross-reactivity with DHT. All samples were measured in duplicate in one assay (sensitivity: 6 pg per tube; intra-assay coefficient of variation 5–8%). For measurement of nitrite (stable metabolic product of NO) levels in the medium, sample aliquots were mixed with an equal volume of Griess reagent, and absorbance was measured at 546 nm (14). Nitrite concentrations were determined relative to a standard curve derived from increasing concentrations of sodium nitrite (1, 2, 20). The cAMP and cGMP were extracted from scraped cells content by ethanol, using a procedure described previously (18). The level of cAMP in cell content of scraped purified Leydig cells was measured with a cAMP EIA kit that permits cAMP measurement with a limit of quantification of 0.1 pmol/ml (at 80% B/B0) and an IC50 of ~0.5 pmol/ml for acetylated cAMP samples (19). The level of cGMP was measured with a cGMP EIA kit that permits cGMP measurement typically with the limit of quantification of 0.07 pmol/ml (at 80% B/B0) for acetylated cGMP samples (2, 19).

**RNA isolation and cDNA synthesis.** Total RNA from purified rat Leydig cells was isolated using an RNasey kit following a protocol recommended by the manufacturer (www.qiagen.com). To eliminate residual genomic DNA, RNA samples were treated with DNase I, and first-strand cDNA was synthesized with a Superscript III reverse transcriptase kit according to the manufacturer’s instructions (www.invitrogen.com) (1, 2, 19, 20).

**Real-time polymerase chain reaction and relative quantification.** The relative expression of the genes was quantitated by real-time polymerase chain reaction (PCR), and two types of chemistries were used to detect PCR products: SYBR Green-based and TLDA (TaqMan Low Density)-based detection in an ABI Prism 7900HT Sequence Detection System (www.appliedbiosystems.com).

**SYBR green.** The relative expression of the genes by use of SYBR Green for amplicon detection was done as previously described (2, 4, 20). Standard PCR settings were used in the presence of specific primers designed by using softver Primer Express 3.0 (Applied Biosystems) and full gene sequences from NCBI Entrez Nucleotide database (www.ncbi.nlm.nih.gov/sites/entrez). The primer sequences used for real-time PCR analysis are given in Table 1 (for steroidogenic machinery components and Actb) and Table 2 (for NO-cGMP signaling elements) in the supplemental material. Relative quantification of each gene was done in duplicate, twice for each of three independent in vivo experiments. The relative quantification of gene expression was calculated using ABI 7900HT SDS and RQ Manager Software.

**TLDA Rat Phosphodiesterase Panel Assay.** The expressions of the genes for PDEs in Leydig cells obtained from control and experimental rats were analyzed in relative quantification real-time PCR by using the TLDA Rat Phosphodiesterase Panel Assay as described previously (20). Each sample was done in duplicate or triplicate, three times for each gene, for each of three independent in vivo experiments. The relative quantification of gene expression was calculated using ABI 7900HT SDS and RQ Manager Software.

**Protein extraction and western blot analysis.** After incubation, Leydig cells (3 × 10^6/well) were lysed as described previously (1, 2, 19, 20). Immunodetection of the StAR protein was performed using the antisera for StAR protein (dilution 1:500; 7, 15). The level of NOS2 was detected by purified anti-mouse antibody against NOS2 (dilution 1:1,000), while GUCY1 subunits were immunodetected with purified rabbit polyclonal antibodies against a1 or b1 subunits of GUCY1 (dilution 1:1,000). PKG1 was detected using rabbit polyclonal antibody recognizing type a/b isoforms of PKG1, while PDE5 was detected with rabbit polyclonal antibody against PDE5 (dilution 1:5000; 26; 1: 1,000, commercial). Actin was detected using an actin detection kit (dilution 1:5,000). The immunoreactive bands were analyzed as two-dimensional images using the Image J (version 1.32; www.rsbo.info.nih.gov/jj/download.html). The optical density (OD) of images is expressed as volume (OD × area) adjusted for the background, which gives arbitrary units of adjusted volume.

**Statistical analysis.** For in vivo studies, the results represent group means ± SE of individual variation from three independent experiments (8 rats per group per experiment). For ex vivo measurement, data represent means ± SE from three to five independent replicates. The results from each experiment were analyzed by Mann-Whitney’s unpaired nonparametric two-tailed test (for two-point data experiments) or, for group comparison, a one-way ANOVA followed by the Student-Newman-Keuls multiple range test.
RESULTS

Effects of prolonged treatment with sildenafil on testosterone, cAMP/cGMP, and nitrite production by Leydig cells. In adult male rats, sildenafil treatment for 30 days caused a moderate but significant increase in serum T+DHT levels (Fig. 1A). Purified Leydig cells derived from sildenafil-treated rats had increased basal testosterone production (Fig. 1B) and higher cAMP level (Fig. 1C). On the other hand, basal NO production by purified Leydig cells from sildenafil-treated animals was decreased (Fig. 1D) following by unchanged cGMP levels (Fig. 1E).

Modulation of Leydig cells steroidogenic activity in sildenafil-treated rats. To investigate the effects of prolonged PDE5 inhibition on Leydig cell steroidogenic capacity, cells were challenged with increasing concentrations of hCG. Leydig cells derived from sildenafil-treated rats exhibited a greater response upon hCG stimulation. This was illustrated by an enhanced dose-dependent increase of testosterone production (Fig. 2A) and cAMP accumulation (Fig. 2B). The expression of specific elements of steroidogenic machinery was analyzed by extracting mRNA from purified Leydig cells obtained from controls and sildenafil-treated male rats. RQ-PCR analysis revealed that LH receptor (Lhr), steroidogenic factor 1 (Sf1), scavenger receptor class B type 1 (Scarb1), Tsps, Star, Cyp11a, Hsd3b, and aromatase (Cyp19), androgen receptor (Ar), and estrogen receptor 1 (Esra1) expressions were unaffected after 30 days of PDE5 inhibition, while only Cyp17 expression was increased (Fig. 2C). Considering the importance of cyclic nucleotides, especially cAMP, in initiation of testosterone synthesis, it was of interest to analyze the PDE expression in the same samples. For that purpose we applied the commercial TLDA Rat Phosphodiesterase Gene Signature Panel. Results showed that expression of Pde4a, which specifically degrades cAMP in Leydig cells, was decreased by sildenafil treatment, whereas other cAMP-specific PDEs, Pde4b, Pde4d, Pde7a, Pde7b, Pde8a and Pde8b, remained unchanged (Fig. 2D).

NO-cGMP signaling pathway in Leydig cells from sildenafil-treated rats. The expression of specific components of NO-cGMP signaling pathway was analyzed by extracting mRNA from purified Leydig cells obtained from controls and sildenafil-treated male rats. Gene and protein expression levels were accessed by RQ-PCR and Western blot, respectively.

Results of real-time PCR analysis revealed that Nos2 expression was decreased in sildenafil treatments, whereas Nos3 was not significantly changed (Fig. 3A). The Nos1 transcript was below detectable level (Ct > 30 cycles), so it was omitted from the present work. Additionally, sildenafil treatment increased expression of Gucy1a1 as well as Gucy1b1 subunits, whereas Pkg1 and Pde5 were not affected (Fig. 3A). TLDA showed that mRNA of Pde6c highly selective for cGMP hydrolysis was decreased by sildenafil treatment, whereas Pde6d and Pde9a were not affected (Fig. 3F). Additionally, regarding PDEs with dual substrate affinity, Pde1a was decreased (Fig. 3G) in the presence of unchanged mRNA expression of Pde1c, Pde2a, Pde3a, Pde3b, and Pde10a (Fig. 3G).

Western blot analysis confirmed an inhibitory effect sildenafil treatment on NOS2 expression (Fig. 3B) and a stimulatory effect on GUCY1 (Fig. 3C). In the same samples, levels of PDE5 (Fig. 3D) as well as PKG1 (Fig. 3E) protein levels were unaffected.

Coordinative actions of cAMP/cGMP on Star activation in Leydig cells from sildenafil treated rats. To study the functional significance of sildenafil-increased GUCY1 expression, Leydig cells derived from sildenafil-treated and control rats were incubated with increasing concentrations of NO donor (DPTA). Since it is well established that NO in higher concentrations directly suppresses Leydig cells in vitro by inhibition of heme-containing steroidogenic enzymes (9, 10, 26), to

Fig. 1. Prolonged sildenafil treatment affects testosterone and messenger production by Leydig cells. Rats were treated daily with sildenafil (1.25 mg/kg body wt) for 30 days. A: serum testosterone levels were determined by RIA. Primary culture of Leydig cells (3 × 10⁶/well) were incubated for 2 h, and testosterone (B) as well as nitrite (D) accumulation were determined in incubation medium. Cells were scraped, and cyclic nucleotides extracted by ethanol and cAMP (C) as well as cGMP (E) levels were determined by RIA. Data bars are group means ± SE of 3 independent experiments. Statistical significance between controls and experimental groups: *P < 0.05.

Fig. 2. Steroidogenic capacity of Leydig cells obtained from sildenafil-treated rats. A: Leydig cells (3 × 10⁶/well) were incubated in the presence of increasing concentration of hCG (0, 0.5, 5 and 50 ng/ml) for 2 h and testosterone accumulation was measured in incubation medium. B: cells were incubated with increasing concentrations of NO donor (DPTA). Since it is well established that NO in higher concentrations directly suppresses Leydig cells in vitro by inhibition of heme-containing steroidogenic enzymes (9, 10, 26), to
reduce the direct inhibitory effect of NO on hormone production, cells were exposed in a dose range to 10 μM DPTA. Such a treatment led to a concentration-dependent increase in cGMP production, which was higher in Leydig cells derived from sildenafil-treated rats (Fig. 4B). The androgen production followed the cGMP pattern (Fig. 4A). In the same samples, sildenafil treatment caused greater accumulation of intracellular cAMP independently of NO stimulation (Fig. 4C). These data taken together, in a stimulatory dose range of DPTA, testosterone correlates much more strongly with cGMP ($R^2 = 0.983$, $P < 0.05$) than with cAMP ($R^2 = 0.693$, not significant).

DISCUSSION

Results from this study have shown that prolonged PDE5 inhibition in vivo stimulated Leydig cells steroidogenesis and increased circulating level of testosterone due to coordinative stimulatory effect of cAMP and cGMP action. Two lines of evidence indicated that prolonged PDE5 inhibition changed the
expression/activity pattern of both cAMP and cGMP signaling pathway elements. First, in the Leydig cells obtained from sildenafil-treated rats, the intracellular cAMP level was increased due to inhibition of Pde4a expression. Second, prolonged sildenafil treatment disturbed NO-cGMP signaling in Leydig cells via inhibition of NOS2 and stimulation of GUCY1 expression. Finally, both signaling pathways converged on StAR protein, stimulating the first step in steroidogenesis to enhance testosterone production.

It is well known that Leydig cell androgenesis depends on a rise at the intracellular level of cyclic nucleotides, especially cAMP, and that its fate, once synthesized, is controlled by catabolic enzymes (36). Results from our study show that adult rat Leydig cells express transcripts for several cAMP-specific Pde genes such as Pde4a, Pde4b, Pde4d, Pde7a, Pde7b, Pde8a, and Pde8b, presumably most of them contributing to Leydig cell response to LHR-cAMP signaling. Decreased expression of Pde4a in Leydig cells obtained from sildenafil-treated rats and consequent increased cAMP accumulation indicates its participation in the control of the relevant cAMP pool(s). Moreover, augmented stimulation of cAMP production in response to hCG stimulation was observed in Leydig cells derived from sildenafil-treated rats. Increased cAMP accumulation could act on PKA to coordinate the transcription of genes required for steroid hormone biosynthesis, including StAR protein, Cyp11a1, and Cyp17a1 (22, 37). Our results showed enhanced Cyp17a1 expression in Leydig cells isolated from sildenafil-treated rats, while the expression for StAR and Cyp11a1 remained unchanged. At present, it is difficult to explain such selective stimulation, since multiple transcription factors, including SF-1, GATA-6, and sterol-regulatory binding protein-1, can be recruited to the promoter during activated Cyp17a1 transcription (34, 37). Although expression of mRNA transcripts for StAR protein was not significantly changed, sildenafil treatment increased the level of mature StAR protein, which was further potentiated in hCG-supported steroidogenesis. At present time, it is difficult to explain the discrepancy observed between the mRNA and protein levels due to the complex mechanisms governing STAR mRNA levels, which include the evidence that some aspects of regulation of STAR expression may occur at the posttranscriptional level (27). Since the StAR protein mediates the rate-limiting step in steroidogenesis, its expression must be finely regulated for its primary expression in steroidogenic cells. Furthermore, StAR gene expression must be able to respond rapidly to various signals that regulate steroidogenesis. Most likely combinatorial events regulate StAR transcription and may affect StAR transcription at different levels. Although the regulation of the StAR gene has been the focus of intense study, the precise mechanism of StAR gene activation remains to be determined, and regulation of the StAR gene appears to be a complex process involving a number of different transcription factors (27). The importance of StAR protein for transport of cholesterol through mitochondrial membranes needed for initiation of steroidogenesis is well illustrated (36). The rise at the intracellular level of cAMP activates phosphorylation of this protein by PKA and initiates the post- or cotranslational events responsible for the occurrence of a mature StAR form that increases its biological activity (7, 21).

In addition to steroidogenic machinery, prolonged sildenafil treatment also affects NO-cGMP signaling in Leydig cells. Our results showed reduced NOS2 expression followed by declined NO production. In the same system, enhanced expression of GUCY1 was observed. In untreated Leydig cells basal GUCY1 activity is relatively low, and PDE5 has little impact under these conditions. In presence of the NO donor DPTA, the activity of GUCY1 was several time higher and was further potentiated by chronic PDE5 inhibition. Since NO from neighboring cells contributes to local regulation of testosterone production (40), augmented GUCY1 could potentiate the paracrine effect of NO. Increased level of GUCY1 in Leydig cells derived from sildenafil-treated rats makes them more sensitive to NO stimulation in terms of increased de novo synthesis of cGMP. It has been shown that in this signaling scenario cGMP activation of PKG1 promotes phosphorylation and consequent activation of StAR protein, leading to increased steroidogenesis (1, 13). These findings helped to further progress in our understanding of molecular events in Leydig cells obtained from sildenafil-treated rats. In these cells, an increased level of mature StAR protein was observed after NO stimulation even in vivo. This was additionally potentiated with hCG-dependent elevation of cAMP. According to results from this study, it seems that both cGMP and cAMP signaling act synergistically and both converge on StAR protein to enhance testosterone production.

In the case of increased NO-dependent GUCY1 activity, decreased expression of cGMP-specific PDEs could be the mechanism that promotes a stimulatory effect of cGMP on Leydig cell steroidogenesis. Eight families of PDEs hydrolyze cGMP with different efficiencies. These eight families are separated into two groups: the cGMP-specific group and the dual-substrate specificity group. Our results indicate that adult rat Leydig cells express mRNAs of Pde5a, Pde6c, Pde6d, and Pde9a from the first group. Sildenafil treatment significantly reduced Pde6c mRNA level in Leydig cells without any effect on Pde5a expression. The reason for such selective inhibition of PDE expression could be compartmentalization of cGMP hydrolytic regulation (12); so the inhibition of specific PDE may result in different targeted effects from those derived by cGMP activation. In addition, sildenafil is a most specific competitive inhibitor for PDE5, but, because of structural similarity of enzyme active site, it also inhibit the photoreceptor PDE6 in the nanomolar range of concentration (3). From the second group of PDEs, with dual-substrate specificity, Leydig cells express Pde1a, Pde1c, Pde2a, Pde3a, Pde3b, and Pde10a. Among them, only Pde1a was inhibited by sildenafil treatment. Ca^2+ /calmodulin-stimulated PDE1 has been shown to be important for the regulation of cGMP level in vascular cells (29). PDE1A1 enzyme activity, protein level, and mRNA expression are selectively upregulated in the nitrite-tolerant rat model (17). Since Leydig cells obtained from sildenafil-treated rats produced lower amounts of NO, lowering PDE1A1 expression could be a consequence of decreased NO production.

The present results support the hypothesis of a dual stimulatory effect of cGMP- and cAMP-mediated signals in Leydig cells and suggests that the effects of PDE5 inhibitors in addition to changed NO-cGMP signaling may also include actions of the cAMP second messenger system. The exact mechanism by which such an interaction occurs is not clear, but it involves decreased expression of the cAMP-specific PDE4a by the inhibition of PDE5. This will, in turn, lead to
increasing levels of cAMP, which promote the interaction of cAMP with PKA followed by activation of StAR protein and the steroidogenic cascade (36). Increased GUCY1 capacity in Leydig cells from sildenafil-treated rats followed by enhanced NO-dependent activation of GUCY1 exhibited an additive effect with cAMP-dependent modification of StAR protein indicates the existence of two mechanisms focused on StAR protein to stimulate androgen production. Our results of decreased expression of cGMP-specific Pde6c and Pde1a with dual selectivity in addition to Pde4a in sildenafil-treated Leydig cells could be mechanism that promotes stimulatory effect of cyclic nucleotides on Leydig cells steroidogenesis.

In addition, it is noteworthy to speculate about the possible effects of sildenafil citrate on testicular blood flow and its possible effect on testicular steroidogenesis. Our preliminary unpublished results showed increased volume of testicular interstitial fluid and testosterone production following the acute, 2-h treatment with Viagra. This effect is most probably a consequence of increased blood flow following sildenafil treatment, and further studies in this subject are required.

Finally, the physiological significance of the results presented was suggested by the increased serum testosterone level following prolonged sildenafil treatment. A similar effect was reported with two different types of PDE5 inhibitors (sildenafil and tadalafil) applied to men who suffered from erectile dysfunction (5). In addition, it has been shown recently that frequent low-dose use of sildenafil and/or tadalafil combined with testosterone has a pronounced antiapoptotic effect on the cavernous tissues of diabetic rats (23). Accordingly the importance of testosterone in maintaining erectile function is clinically manifested in disorders such as the metabolic syndrome, in which the hypogonadal state is frequently accompanied by erectile and sexual dysfunction.

ACKNOWLEDGMENTS

We are very grateful to Prof. Sergei Rybalkin (University of Washington, Seattle, WA) for the very kind donation of PDE5 antibody, Profs. Douglas Stocco (Texas Tech University) and Dale Hales (University of Illinois) for the generous and continuous donation of StAR antisera. We appreciate Prof. Gordon Nwosuender (Colorado State University) for supplying antibodies for RIA analysis.

GRANTS

This work was supported by the Serbian Ministry of Science and Technological Development (Grant No. 143055), Autonomic Province of Vojvodina (Grant No. 0707), and a grant to S. A. Andric from the Serbian Ministry of Science and Technological Development (Grant No. 451-03-00275/2009-02).

DISCLOSURES

No conflicts of interest are reported by the authors.

REFERENCES


