Volume 8, Issue 3, 2018, 3237 - 3240 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article **Open Access Journal**

Received: 23.04.2018 / Revised: 28.05.2018 / Accepted: 10.06.2018 / Published on-line: 15.06.2018

Examination of the effects of different phosphodiesterase type 5 enzyme inhibitors on the isolated rat myometrium contraction

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ABSTRACT

The present study aims to explore the effects of the phosphodiesterase inhibitors tadalafil and vardenafil on the spontaneous contractions of isolated rat myometrium.The study included 45 virgin female rats weighing between 250 and 260 g. Two different agents were used in two different tissue cross-sections taken from each animal. Spontaneous contractions as well as the contractions following tadalafil and vardenafil applications (200, 400, and 600 µl) in the tissue preparations, were evaluated in 10 minute periods in terms of frequency, amplitude, and the area under the contraction curve. Isometric contractions were measured and evaluated on the oscillographic records. The highest tadalafil dose (600 µl) significantly reduced frequency in comparison to control values ($p<0.01$). Vardenafil did not produce any effect on contraction frequency. When the effects of the agents on contraction amplitude were evaluated, the highest dose of tadalafil was again found to lead to a significant drop in the amplitude ($p<0.05$). The same dose of tadalafil was also seen to markedly decrease the area under the curve ($p<0.05$).Results of the study indicate that tadalafil markedly inhibits the frequency, amplitude, and area under the contraction curve, all of which are isolated myometrial contraction parameters in rats, in a dose-dependent manner. However, vardenafil did not produce any effect on these parameters.

Keywords: *Myometrial contractions; tadalafil;vardenafil; rat.*

1. INTRODUCTION

In order for the induction of labor, several cellular and molecular changes must take place in the fetus, placenta, cervix and uterus tissues, including the maturation of the cervix, myometrial contractions, and tear of fetal membranes [1,2]. These molecular changes are made possible by several cellular secondary precursors and the activation of ion channels.

Cyclic guanosine 3'5' monophosphate (cGMP), an intracellular secondary precursor, has a critical part in the regulation of common physiological events such as smooth muscle relaxation, neutrophil degranulation, inhibition of thrombocyte aggregation, induction of visual signal conduction, spermatozoa motility, and germ cell production [3]. Intracellular cGMP levels are determined by the balance between the synthesis of cGMP by guanylylcyclase and its destruction by specific phosphodiesterases. Presently, at least 11 different subtypes of the phosphodiesterase enzyme family have been reported and their regulator characteristics and inhibitor profiles have been described using molecular techniques [4]. The inhibition of these enzymes leads to an increase in intracellular cGMP levels, thereby affecting the physiological functions of tissues.

Although the role of cGMP in sustaining pregnancy and initiating labor is still open to debate, L-arginine-nitric oxide (NO)-cGMP pathway is accepted to contribute to the stability of the uterus throughout pregnancy and it is believed that the reduced susceptibility of uterus to this system at term is among the mechanisms involved in initiating labor [5]. However, there are studies suggesting that cGMP is not involved in the inhibition of uterus contractions by this system and instead, the inhibition is due to mechanisms including the activation of adenosine triphosphate (ATP) and calcium-sensitive K+ channels of nitric oxide [6]. Still, there are other studies reporting that cGMP serves as a second precursor in the uterus smooth muscle, just like it does in skeletal muscles and other smooth muscles, and is involved in NO-mediated control of myometrial contractility [7]. Nitric oxide is bound to guanylatecyclase, elevates cGMP levels and causes the smooth muscles to relax. Synthesized locally in the uterus, both NO and cGMP were found to markedly inhibit contractile activity in the rat uterus [8].

Vardenafil (Levitra) is a phosphodiesterase type 5 enzyme inhibitor9. It reaches its peak level in the blood in 0,7-0,9 hours. Used at doses of 5, 10, and 20 mg, vardenafil has a success rate of about 80% and appears superior to other PDE5 inhibitors [9-10].
Tadalafil, another phosphodiesterase type 5 enzyme inhibitor with a different structure causes relaxation of the cavernous tissue [11]. Tadalafil is absorbed into the blood circulation slower than vardenafil and it reaches its peak level in 2 hours. According to clinical data, the half-life of tadalafil (the time it takes for the blood level of the substance to be reduced to half) is longer than that of its counterparts (17,5 hours) and it maintains its efficiency for 36 hours [12].

Although the effects of tadalafil and vardenafil on smooth muscle contraction in other tissues have been studied, their effects on the contraction of the myometrium smooth muscle tissue are not known. This comparative experimental study shall identify the inhibiting effects of the concerned agents on uterus smooth muscle and explore the possibility of achieving higher efficiency at a

lower dose through combined use in order to procure an advantage in terms of the side effect profile.

The present study aims to comparatively evaluate the effects of vardenafil and tadalafil, both of which are phosphodiesterase type 5 enzyme inhibitors, on spontaneous contractions in the isolated rat myometrium.

2. EXPERIMENTAL SECTION

2.1. Animal Material and Preparation of Uterus Preparations. The study was carried out in the Physiology Department Laboratory of Fırat University Medical School. After the study protocol was approved by the local ethics committee, 45 adult female virgin rats of Wistar type, weighing 250-260 g, were included in the study. The animals were obtained based on the number of daily experiments. Care was taken to make sure that the animals were not younger than eight weeks and in the diestrus phase. After the dislocation of the cervix, the abdomen was opened, internal organs were removed and two uterus horns located between the ovaries and uterus body were carefully dissected. After the dissection, the uterus was put into a petri dish containing Krebs solution. By cleaning the fat tissue around the uterus, the preparation process was started. The preparations were 1x0,2x0,2 cm in size. Uterus tubes were dissected longitudinally. The lower end was tied as a ring and connected to the double-jacketed isolated organ bath instrument containing Krebs solution and the upper end was tied in a knot and connected to the displacement transducer.

2.2. Experiment Procedure. Uterus preparations from 10-week-old Wistar albino rats were placed in 1x0,2x0,2 cm Petri dishes containing Krebs solution. The preparations were mounted vertically on the organ bath by fixating the lower end to the lower side of the isolated organ bath in the form of a ring using 2.0 silk and the upper end to the isometric force displacement transducer with a straight knot. Before starting the experiment, the experiment system was standardized. A 1gr. weight was mounted on the force transducer to set the values in the software according to this weight and the system was calibrated to show 1 gr. tension. After the uterus preparations were suspended on the isolated organ bath, the micro screw was moved to apply a 1-gram resting tension to the preparation and 30 minutes were left to pass for the myometrium to adapt to this tension. Preparations which did not display regular contractions were excluded from the experimental study. During this period, the uterus preparation was bathed in Krebs solution every 10 minutes. At the end of the waiting period, the contractions were recorded for 10 minutes and the records were used for control purposes.

At the end of the period, 200 microliters of Tadalafil and Vardenafil doses were applied to the uterus preparations in the isolated organ bath. After observing the recordings for 10 minutes, the effects of the agents on the frequency, amplitude and the area under the curve of contractions were evaluated. The same preparations were applied 400 microliters of Tadalafil and Vardenafil doses as of minute 20 and 600 microliters of Tadalafil and Vardenafil doses as of minute 30. The effects of the agents on the frequency, amplitude and the area under the curve of contractions were assessed in the same way.

2.3. Isolated Organ Bath. The system consists of a chamber where the uterus preparation is placed, a reservoir holding the solution, tubes where the Krebs solution flows and is discarded from, and an isometric force transducer. Thanks to its jacketed structure, the whole system is heated from outside using distilled water kept at 37^{o}C by a thermostated circulating pump.

The Krebs solution in the isolated organ bath was gassed with a mixture of 95% O_2 and 5% CO_2. Isometric force displacement transducer (MAY IOBS 99) converts the force produced by the contraction of the myometrial band to bioelectrical potentials. The bioelectrical potentials obtained as such are transferred to the amplifier, amplified, and then recorded on the computer through its analog/digital main unit. The data are stored by Biopac 10.0 software.

2.4. Solutions. Krebs solution with a pH value of 7,4 was used in this study. The content of the solution is the same as the solution used to record isolated rat myometrium contractions in the literature [13]. The pH of the Krebs solution was measured daily and adjusted to 7,4, when necessary by adding distilled water. While preparing the solution, calcium chloride and magnesium chloride were dissolved separately and then added to the total solution. In the meantime, the solution was constantly shaken to ensure dissolution of the added chemical content and to prevent precipitation.

2.4. Statistical methods. All data are presented as a mean ± standard error. SPSS 10.0 and Origin 5.0 software packages were used in statistical evaluations and graphing, respectively. Student'st,test was employed in the two-way comparisons of amplitude and frequency data and Kruskal-Wallis variance analysis in three-way comparisons. The areas under the contraction curve were calculated as percentage units. Level of significance was set at $p<0,05$.

4. RESULTS AND DISCUSSION

Of the 45 myometrium cross-sections in the study, 38 were observed to have regular contractions in the period of adaptation to resting tension; 5 muscles which had irregular contractions and 2 which did not show any spontaneous activity were excluded from the study.When the effects of tadalafil on myometrial contractions were examined, it was seen that doses of 200 and 400 µl did not have any effect on contractions, while the administration of 600 µl led to a significant decrease in the frequency of contractions in comparison to the frequency of spontaneous contractions (Table 1, $p<0,01$).

Table 1. Effects of Tadalafil on Contraction Frequency, Amplitude and the Area under the Curve.

	Spontaneous	200 µl	400 µl	600 µl
Frequency (Contraction Frequency/10 min.)	7.00±3.00	5.50±2.09	4.44±2.35	2.77±2.22*
Amplitude (mgr)	1954.10±503.20	1690.40±562.60	1523.00±591.70	1214.30±716.30**
Area under the Curve	2932±486	2438±332	2072±340	1445±211**

*P<0.01; ** P<0.05

An examination of the effects of vardenafil on contraction frequency revealed that none of the doses used in the study produced any effect on contractions (Table 2).

An evaluation of the effects of tadalafil and vardenafil on contraction amplitude demonstrated that 600 µl tadalafil markedly reduced the contraction amplitude (Table 1, p<0,05), while other doses of the same agent did not produce any effect. In the same vein, none of the vardenafil doses brought about any effect on contraction amplitude (Table 2).

When the effects of the agents on the area under the contraction curve were investigated, tadalafil at the dose of 600 µl was found to reduce the concerned area significantly (Table 1; p<0,05). As for the effects of vardenafil on this parameter, it was seen that none of the three vardenafil doses were effective (Table 2).

Table 2. Effects of Vardenafil on Contraction Frequency, Amplitude and the Area under the Curve.

	Spontaneous	200 µl	400 µl	600 µl
Frequency (Contraction Frequency/10 min)	6.83±2.93	6.00±3.42	5.44±3.97	5.44±2.85
Amplitude (mgr)	2208.10±1015	1884.40±974	1472.00±1062	1221.30±916.30
Area under the Curve	6932±525	5161±407	4781±396	3823±360

An overall evaluation of the study results suggests that the phosphodiesterase inhibitor tadalafil inhibits spontaneous myometrial contractions in a dose-dependent manner. This inhibition is most evident with the highest dose of 600 µl. Vardenafil, another phosphodiesterase inhibitor, however, did not produce any effect on the studied parameters at any of the doses used.

Besides being destroyed by phosphodiesterases, cGMP is also exported from the cells. This export occurs through an ATP-dependent export pump for cGMP. The concerned pump is also known as multi-drug resistance-associated protein 5. This protein was described in the smooth muscle of the genitourinary system with phosphodiesterase 5 (PDE5) [14]. PDE inhibitors strongly suppress this multidrug protein. Therefore, the binding of a PDE inhibitor like sildenafil to a multidrug resistance-associated protein forms another pathway for the relaxation of corpus cavernosum muscle.

cGMP is accepted to interact with kinase G to affect gap junction and ion channels, reducing calcium intake into the cell and cytoplasmic Ca levels. Consequently, it inactivates myosinkinase and causes the relaxation of smooth muscle cells [11]. However, it was reported that, in human cavernosum muscle, sildenafil did not activate potassium channels activated by calcium and that its muscle-relaxing effect might not have been due to the opening of these channels [15].

It was suggested in another study that phosphodiesterase 5 inhibitors like sildenafil, tadalafil,andvardenafil led to the relaxation of corpus cavernosum not through their inhibiting activity, but by regulating calcium mobilization. It was also reported that the differences in the structures of vardenafil and tadalafil might influence calcium intake in different ways [11].

Additionally, tadalafil, which is a novel phosphodiesterase type 5 enzyme inhibitor differing in structure from the other members of its group, causes the relaxation of the cavernous tissue11. It takes longer for tadalafil to get into the blood circulation. Clinical data suggest that the half-life of tadalafil (the time it takes for the blood level of the substance to be reduced to half) is longer than that of its rivals (17,5 hours) and that it maintains its efficiency for 36 hours [12]. In the present study, tadalafil was again found to markedly inhibit myometrial contractions, as it did in other tissues.

Vardenafil is another phosphodiesterase type 5 enzyme inhibitor. There are certain pharmacological similarities between vardenafil and tadalafil. Vardenafil reaches its peak level in circulation in 0,7-0,9 hours. Although vardenafil, used at doses of 5, 10 and 20 mg was found to have success rates up to 80% in different tissues in previous studies, it did not cause any significant effect on the myometrium in this study. A similar previous study examined the effect of phosphodiesterase inhibitors sildenafil, tadalafil and vardenafil on contractions in rat anococcygeus muscle. The study reported that PDE5 inhibitors acted by elevating endogenous and exogenous nitric oxide levels and that vardenafil had a stronger effect than sildenafil and tadalafil in this respect [7,16]. It was noted in the same study that *in vitro*vardenafil was more potent than sildenafil and tadalafil in preventing the destruction of cGMP. In another study comparing tadalafil and vardenafil, Toque et al.16 established that tadalafil had a more powerful calcium retention effect and thus, by reducing calcium mobilization, it served as a stronger vasodilator in pulmonary arteries. A similar result was reported by Ghofrani et al. [17]. However, in a study carried out on rat aorta, it was found that, in addition to elevating the cGMP levels in the tissue, vardenafil inhibited PDE5 by affecting calcium retention, while tadalafil and sildenafil did not produce similar effects in the concerned tissue [18].

In a study exploring the effect of the PDE5 inhibitor sildenafil on rat uterus contractions, sildenafil was established to inhibit the contractile effect caused by oxytocic agents and this inhibitive effect was brought about by the action of the agent on cGMP [19].

In our study, PDE5 inhibitors tadalafil and vardenafil were used in isolated myometrium of virgin rats. Our literature review shows that the studies about the effects of these drugs on myometrial contractions were not adequate and focused predominantly on the effects of the drugs on the vascular bed. Although the same doses of the drugs were used, the results were seen to vary. As a matter of fact, the studies cited above reported that PDE5 inhibitors produced different effects in different tissues. The results of the present study demonstrate that the phosphodiesterase inhibitors tadalafil and vardenafil had varying effects on spontaneous contractions in the rat myometrium. This variation can be attributed to the structural differences between the PDE5 inhibitors and the responses of the concerned tissue to the drug. The results of the study show that tadalafil markedly inhibits the isolated rat myometrial contraction parameters frequency, amplitude and the area under the contraction curve in a dose-

dependent manner. However, vardenafil was not seen to produce similar effects. The results concerning vardenafil can be explained by the relation between dose and duration. Use of different doses and durations in vardenafil administration in future studies may provide new information on this topic.

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