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Peptidoglycan modulates rat myometrial contractility via Ca^{2+} release from SR

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The aim of current trial was to investigate the SR role in the peptidoglycan elicited alteration of myometrial contractility. For the study we used peptidoglycan from Staphylococcus aureus. In order to assess the peptidoglycan's effect on myometrial contractility under different conditions we used the method of tensometry and took into account the average amplitude, frequency and the area under the curve of the myometrial contractions. The results of our experiments confirmed our suggestion that peptidoglycan activates myometrial contractility due to the SR depletion via IP3 receptors activation as well as the amplification of transmembrane Ca^{2+} influx. Under peptidoglycan the amplitude of myometrial contractions increased by nearly 7,0 ± 0.346% [n=10] compared to the control. The area under the curve increased by nearly 30,0 ± 0.256% due to both the amplitude and the duration of contractions. In our study we demonstrated that peptidoglycan makes the myometrial strip to contract even in the Ca^{2+}-free environment throughout a few minutes. Also we demonstrated that phospholipase C blockade with U^{73122} doesn't prevent entirely the stimulation of myometrial contractions by peptidoglycan. Phospholipase C blockade resulted in statistically unreliable increase in the amplitude of the peptydoglycan-stimulated myometrial contractions, their frequency decreased by 47 ± 1.05% and the area under the curve of single contraction decreased by nearly 56 ± 1.2%. But 2APB, a non selective IP3 antagonist, entirely reduced the peptidoglycan-stimulated contractions. We suggested that peptidoglycan acts via several pathways simultaneously and Ca^{2+} release from SR via IP3 receptors activation is among them.

Key words: peptidoglycan; myometrium; myometrial smooth muscle cells; myometrial contractile activity.

INTRODUCTION

Myometrial contractile activity is one of the main factors required for the realization of the female reproductive capacity. Normal contractility is necessary to empty the uterine cavity during the menstrual, for the active transport of sperm from the vagina to the fallopian tubes and for embryo implantation. It determines the tropism of myometrium itself, uteroplacental circulation, safe childbearing and parturition [1, 2]. Pelvic inflammatory diseases of infectious origin are the most common cause of myometrial contractility disorders and infertility [3, 4]. Recent studies demonstrated that not only bacteria but also their structural components, particularly peptidoglycan could lead to pathological conditions. Indeed it provides a pathogenic effect for the female reproductive system among others. Ilievski V. and Hirsch E. had shown that intrauterine administration of peptidoglycan caused preterm birth in pregnant mice [6]. That's why peptidoglycan-caused alteration of myometrial contractility became the deal of our investigation.

Peptidoglycan is an essential cell wall component of almost all bacteria, except mycoplasma. It releases into the milieu throughout the growth, division or destruction of bacteria [7]. Tall-like receptors -2 [TLR-2] are known to recognize peptidoglycan. TLRs are a member of pattern recognition receptors large family. They regulate many inflammatory processes, recognize, and respond to pathogen-associated molecular patterns [8]. They are known to be a functional component of the innate immunity. On the myometrial

myocytes TLR-2 receptors are expressed. [9,10]. Evidently, between the uteral myocytes and peptidoglycan a direct contact occurs. It is known that activation of TL receptors in myometrium contributes to the modification of the innate immune system and initiates an inflammation. Besides them it is shown that TLR-2 activation reduces the chances of implantation success [11]. However, the cellular mechanisms and functional manifestation of TLRs activation influence on myometrial contractility modulation still remain unclear.

It is previously speculated that TLRs activation leads to the Ca^{2+} influx [12] but the character of alteration of myometrial contractions parameters under peptidoglycan in our previous studies [13] led us to an opinion that afforested changes occur through Ca^{2+} release from the intracellular Ca^{2+} depot, namely from the sarcoplasmic reticulum (SR)[14]. Therefore, this study deals with the investigation of the SR role in the peptidoglycan caused alteration of myometrial contractility.

METHODS

The experiments were conducted on myometrial strips from mature Wistar rats weighing 200-250 g in accordance with The European Convention on Bioethics [Strasburg, 1986]. The rats were humanly killed by CO_2 anesthesia followed by decapitation. The uterus was cut up and placed in the heated [37˚], oxygenated [95% O_2 and 5% CO_2] Krebs solution of the following content (mmol/l): NaCl-120.4, KCl - 5.9, $CaCl_2$ - 1.8, $MgCl_2$- 1.2, NaH_2PO_4 - 1.2, $NaHCO_3$ - 15.5, glucose -11.5, PH 7.4. Then longitudinal strips were dissected, 1-3 mm × 5-7 mm, connective tissue and endometrium of samples were removed. Then they were placed in a flow chamber with one end attached to the force transducer and to the fixed stainless steel hook at the other end. The force transducer was connected to the computer via an analog-digital converter. To reveal the peptidoglycan effect on the myometrial contractility we assessed such parameters as the contraction frequency for ten minutes, their average amplitude and the area under the curve of a single contraction. Values are given as mean ± standard error of the mean, "n" is the number of myometrial strips. Differences were considered significant for P±0.05 using the appropriate Student's t test. The comparison was performed with univariate dispersive analysis ANOVA with Bonferonni adjustment.

Peptidoglycan was applied at a dose of 0.003 mg/ml. Strips were left until spontaneous contractions became regular and stable before applying the blockers, Ca^{2+}-free solution and peptidoglycan. U73122 was used as a phospholipase C blocker (10 μM), 2APB - as IP3 blocker (30 μM). All reactive are produced by "Sigma Aldrich" (USA).

RESULTS AND DISCUSSION

Under control conditions isolated myometrial strips were spontaneously active and contracted regularly. We applied peptidoglycan. The results of our experiments demonstrated that it increases the amplitude of contractions by nearly 7,0 ± 0.346% [n=10] compared to the control [Fig. 1a, b]. The area under the curve increased by nearly 30,0 ± 0.256% due to both the amplitude and the duration of contractions. At the same time the contractions frequency decreased by nearly 10,0 ± 0.026% under peptidoglycan. In 85% of cases peptidoglycan leaded to the decline of the basal tone. Throughout all the period of peptydoglycan application this decline reached nearly 3,0 ± 0.58 % of the average amplitude of spontaneous myometrial contractions in control.

Thus, the results of our experiments presented that peptidoglycan causes an activation of myometrial contractility. Moreover, it altered all parameters of nonpregnant myometrial contractions. According to our results peptidoglycan mostly elongated the duration of myometrial contraction, whereas the magnification of their amplitude was less pronounced.

To prove our hypothesis that peptidoglycan causes Ca^{2+}-release from intracellular Ca^{2+}

-stores, we investigated wether peptidoglycan provides its stimulating effect when transmembrane Ca^{2+} influx is impossible. Under regular spontaneous contractions we replaced Krebs solution by nominally Ca^{2+}-free Krebs solution. The contractions gradually reduced. When they stopped entirely we added peptidoglycan to the Ca^{2+}-free Krebs solution. As a result, a few phasic contractions appeared throughout 3 minutes [Fig. 2]. No basal tone decline was observed. We suppose that the contractions continued for a few minutes because of the peptidoglycan-caused SR depletion.

As it is known that activation of ryanodine receptors does not evoke myometrial contractions [15], we evaluated the role of phospholipase C- IP_3 pathway. So we conducted a series of experiments using a phospholipase C blocker U73122 and then IP3 receptor antagonist 2-APB. So under the spontaneous myometrial contractile activity we applied peptidoglycan. It resulted in stimulation of myometrial contractility.

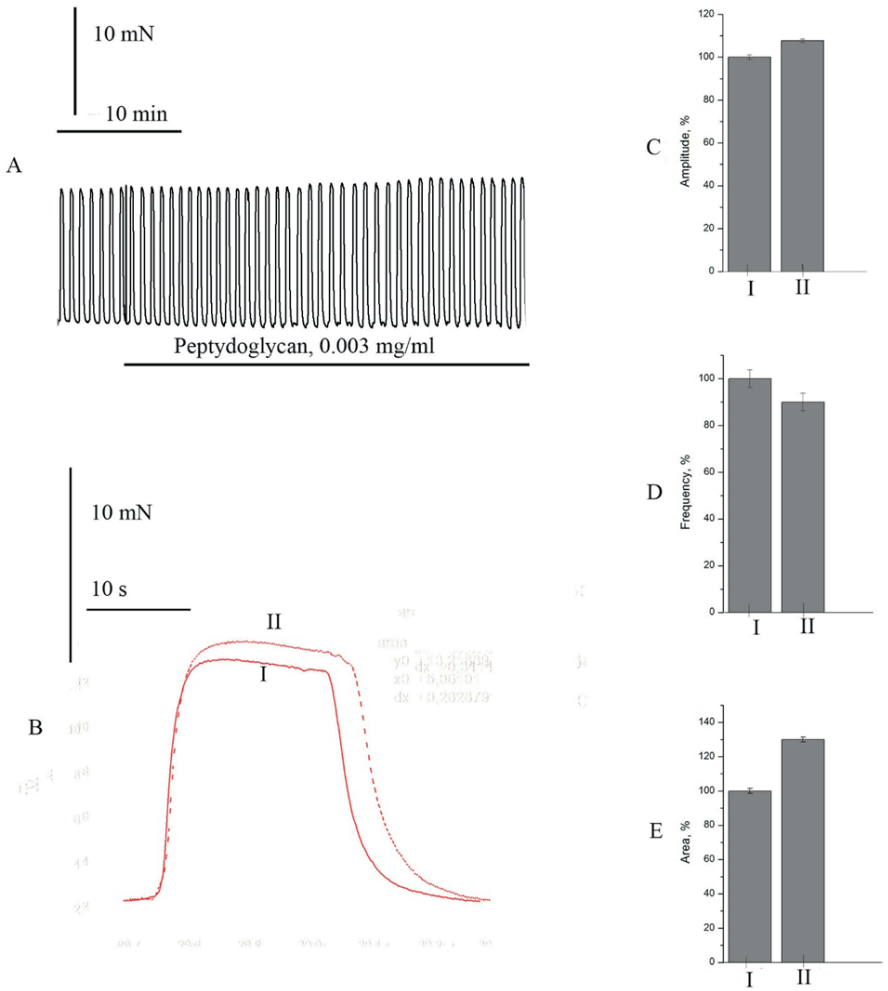

Fig. 1. Typical records showing effects of peptidoglycan on the myometrial contractions parameters.
I - spontaneous myometrial contraction(s), II - myometrial contraction(s) under peptidoglycan.
A. Peptidoglycan increases the amplitude and duration of myometrial contractions but decreases their frequency and basal tone.
B. Peptidoglycan increases both amplitude and duration of myometrial contraction.
C. Under peptidoglycan myometrial contractions amplitude rises.
D. The frequency of myometrial contractions decrease under peptidoglycan by 10%.
E. The area under curve increased by 30% during application of peptidoglycan

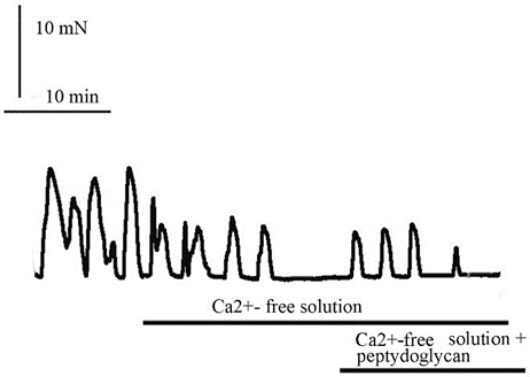

Fig.2. Typical records showing effects of peptidoglycan in a Ca^{2+}-free Krebs solution after spontaneous contractions disappearing under Ca^{2+}-free solution

Afterwards we added peptidoglycan to the Krebs solution with U73122. Phospholipase C blockade resulted in statistically unreliable increase in the amplitude of the peptidoglycan-stimulated myometrial contractions, their frequency decreased by 47 ± 1.05% and the area under the curve of single contraction decreased by nearly 56 ± 1.2% [Fig.3]. Nearly in 35% of samples, the U73122 application didn't result in any changes in the amplitude of the contractions. Thus phospholipase C blockade only partially influenced the peptydoglycan's effect. The reason for this, in our mind, could be the next: peptidoglycan activates myometrial contractility due to the SR depletion as well as the amplification of transmembrane Ca^{2+} influx.

It is known, that all six isotypes of mammalian phospholipase C are G-protein-binded enzymes [16]. Given that in our previous [17] study we demonstrated the link between peptidoglycan's effect and G-protein activation, we propose that Ca^{2+}-depot depletion could be just one of the mechanisms of the peptidoglycan's action.

Phospholipase C is involved in regulation of intracellular Ca^{2+} via mediating the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PI 4,5-P2) to the second messenger molecules inositol 1,4,5-trisphosphate [IP3]. IP3, in turn, regulates the release of stored Ca^{2+} from SR into the cytosol and nucleus [18]. So, the aim of the next series of experiments was to detect if peptidoglycan affects the myometrial spontaneous contractility when it is applicated under IP3 receptors blocker 2-APB. So we applied peptidoglycan, which stimulated the contractions. Then we applied it simultaneously with 2APB. As a result, the contractions gradually decreased and disappeared entirely [Fig.4]. Under IP3 receptor blocker peptidoglycan didn't act as stimulator of myometrial contractility. Unlike U73122, 2-APB decreased all parameters of myometrial contractions.

Such results confirmed our suggestion that peptidoglycan activates myometrial contractility due to the SR depletion via IP3 receptors activation as well as the amplification of transmembrane Ca^{2+} influx. In our study we demonstrated that peptidoglycan makes the myometrial strip to contract even in the Ca^{2+}-free environment. Also we demonstrated that phospholipase C blockade doesn't prevent entirely the stimulation of myometrial contractions by peptidoglycan, but IP3 receptors blockade does. We suggested that peptidoglycan acts via several pathways simultaneously and therefore the blockade of only one of them wouldn't give any preventive effect.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

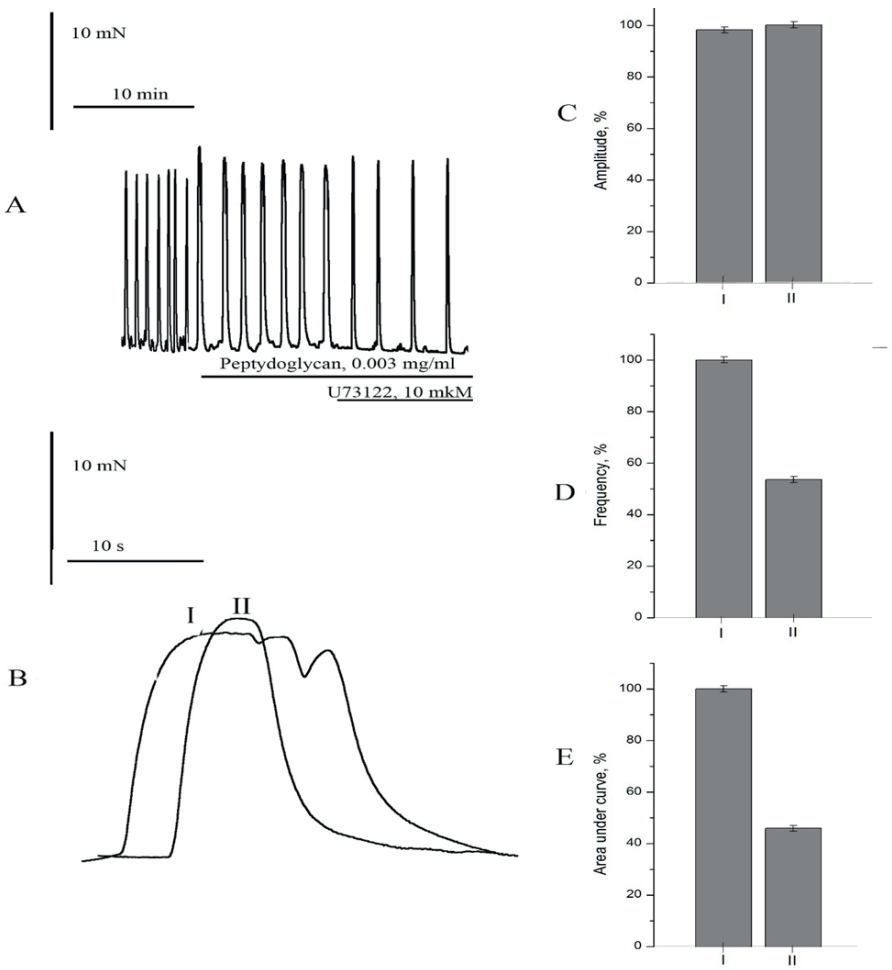

Fig.3. Typical records showing U73122 to decrease the duration and the frequency of the peptidoglycan stimulated myometrial contractility.
I - peptidoglycan stimulated myometrial contraction(s), II - myometrial contraction(s) under U73122.
A. U73122 insignificantly increased the amplitude, but decreased the duration and frequency of the peptidoglycan stimulated myometrial contractility.
B. U73122 decreased the duration of the peptidoglycan stimulated myometrial contraction.
C. Under U73122 the peptidoglycan stimulated myometrial contractility amplitude raised by 2%.
D. The frequency of myometrial contractions decrease under peptidoglycan by 10%.
E. The area under curve decreased by 57% during application of U73122

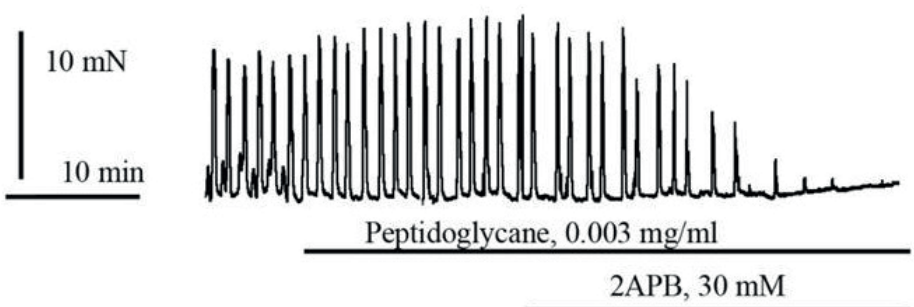

Fig.4. Application of 2APB results in reducing of peptidoglycan - induced contractions

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ПЕПТИДОГЛИКАН МОДУЛИРУЕТ СОКРАТИТЕЛЬНУЮ АКТИВНОСТЬ МИОМЕТРИЯ ПОСРЕДСТВОМ ВЫСВОБОЖДЕНИЯ Ca^{2+} ИЗ САРКОПЛАЗМАТИЧЕСКОГО РЕТИКУЛУМА

Целью данного исследования было определение, действительно ли пептидогликан способствует высвобождению Ca^{2+} из саркоплазматического ретикулума (СР) гладкомышечных клеток миометрия, и если да, то на какое именно звено этого патогенетического пути он действует. Использовали пептидогликан клеточной стенки золотистого стафилококка. Сократительную активность миометрия исследовали на 40 самках крыс методом тензометрии, полученные результаты оценивали по следующим параметрам: средняя амплитуда, частота и площадь под кривой сокращений миометрия. Результаты экспериментов подтвердили предположение, что пептидогликан активирует сократимость миометрия вследствие истощения СР за счет активации рецептора инозитолтрифосфата (ИФ3), а также увеличение трансмембранного транспорта Ca^{2+}. При аппликации пептидогликана полоска миометрия сокращалась даже в среде без Ca^{2+}. Блокирование фосфолипазы С не полностью нивелировало стимулирующее действие пептидогликана на сокращения миометрия. Оно привело к статистически недостоверному увеличению амплитуды сокращений миометрия, вызванных пептидогликаном, их частота уменьшилась на 47 ± 1,05%, а площадь под кривой одиночного сокращения уменьшилась почти на 56 ± 1,2%. А вот на фоне действия блокатора ИФ3- рецепторов действие пептидогликана отсутствовала. Мы предположили, что эффект пептидогликана обусловлен одновременно несколькими путями, и высвобождение кальция из СР при активации ИФ3 -рецепторов - один из них.

Ключевые слова: пептидогликан; миометрий; гладкие мышечные клетки миометрия; сократительная активность миометрия.

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ПЕПТИДОГЛІКАН МОДУЛЮЄ СКОРОТЛИВУ АКТИВНІСТЬ ЧЕРЕЗ ВИВІЛЬНЕННЯ Ca^{2+} ІЗ САРКОПЛАЗМАТИЧНОГО РЕТИКУЛУМА

Метою нашого дослідження було визначити, чи дійсно пептидоглікан сприяє вивільненню Ca^{2+} із саркоплазматичного ретикулуму (СР) гладеньких міоцитів матки, і, якщо так, то на яку саме ланку цього патогенетичного шляху він діє. Використовували пептидоглікан клітинної стінки золотистого стафілококка. Скоротливу активність міометрія досліджували на 40 самицях щурів методом тензометрії, отримані результати оцінювали за такими параметрами, як: середня амплітуда, частота і площа під кривою скорочень міометрія. Результати експериментів підтвердили припущення, що пептидоглікан активує скоротливість міометрія внаслідок виснаження СР за рахунок активації рецепторів інозитол три фосфату(ІФ3), а також збільшення трансмембранного транспорту Ca^{2+}. Під дією пептидоглікану амплітуда скорочень міометрія збільшилась майже на 7,0 ± 0,346% (n = 10) порівняно з контролем. Площа під кривою збільшилася майже на 30,0 ± 0,256% за рахунок амплітуди і тривалості скорочень. При аплікації пептидоглікану смужка міометрія скорочувалася навіть у середовищі без Ca^{2+}. Блокування фосфоліпази С не повністю нівелювала стимулювальну дію пептидоглікану на скорочення міометрія. Вона призвела до статистично недостовірного збільшення амплітуди пептидоглікан-стимульованих скорочень міометрія, їх частота зменшилась на 47 ± 1,05%, а площа під кривою одиничного скорочення зменшилася майже на 56 ± 1,2%. А от на тлі дії блокатора ІФ3- рецепторів дія пептидоглікану була відсутня. Ми припустили, що ефект пептидоглікану обумовлений одночасно кількома шляхами, і вивільнення кальцію із СР при активації ІФ3- рецепторів - один з них.

Ключові слова: пептидоглікан; міометрій; гладенькі м'язові клітини міометрія; скоротлива активність міометрія.

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