Xestospongin C-sensitive calcium stores and angiotensin II-induced myometrial contraction

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Abstract

Objective: *The role of inositol 1,4,5 triphosphate (IP3)-sensitive calcium stores on angiotensin II (AG II)-induced uterine contraction was studied by blocking IP3 receptors with xestospongin C (Xe C). This is for the first time when Xe C is used to evaluate the implication of IP3-sensible intracellular calcium stores, during AG II-induced uterine contraction.* ***Study design:*** *We worked on non pregnant rat uterine strips, comparing contractions to AG II 10-7M, before and after Xe C 10-7M administration.* ***Results:*** *Xe C decreased the AG II-induced contraction with 34.12±11%, the amplitude being more inhibited than the frequency, which was decreased with 18.75±5%. Meantime, Xe C reduced also the amplitude of the automatic oscillations with 38.79±8%, but their frequency was not affected.* ***Conclusions:*** *IP3-sensitive calcium stores have a stronger role on force than on frequency of oscillations during AG II-induced contraction. In basal conditions, these calcium stores are partially responsible for the amplitude of automatic uterine contractions, with no impact on spontaneous depolarizing, since frequency is not decreased by Xe C.*

Keywords: *endoplasmic reticulum calcium stores, IP3, myometrium, angiotensin II, xestospongine C*

IP3-induced intracellular calcium mobilization represents the most important step in angiotensin II-induced uterine contraction and is also involved in spontaneous myometrial activity.

Introduction

Inositol 1,4,5 trisphosphate (IP3), discovered by Berridge in 1983[1], is the first important second messenger, with strong impact on calcium mobilization. It results after hydrolization of membranal phosphatydilinositol 4,5 bisphosphate (PIP2) under phospholipase C (PLC) action (beside IP3, being generated also diacylglycerol - DAG). PLC is activated as the result of binding of an ocytocic to its specific receptor, which is almost always coupled with a G protein (Gq)[2].

IP3 binds to its specific receptors, which are calcium channels, situated on smooth endoplasmic reticulum calcium stores, inducing mobilization of this ion in cytosol.

The mobilized calcium is able to depolarize the cell membrane. As a consequence, L-calcium channels are opened, with massive calcium entry in cell and contraction.

Calcium mobilized from IP3-sensitive stores is also able to open the ryanodine-sensitive calcium stores (Ca_2+-induced - Ca_2+ release - CICR)[3].

The PLC/PIP2/IP3 system is the most important intracellular signaling system initiated by all ocytocics, including angiotensin II (AG II). This peptide induces uterine contraction after binding to AT1-receptors on myometrial smooth muscle cell membrane[4].

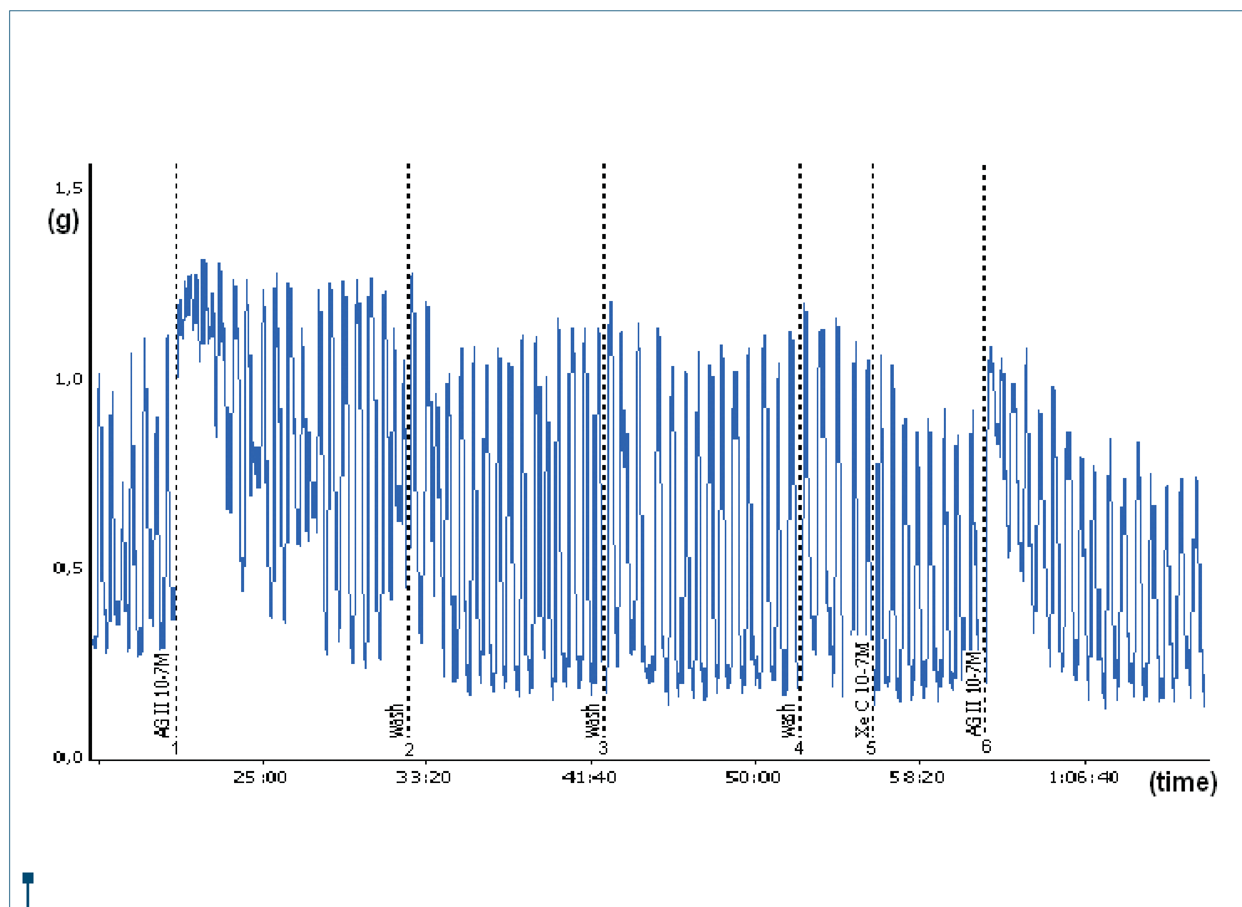

Figure 1. Effect of Xe C 10-7M on AG II 10-7M induced myometrial contraction

Xe C is an antagonist of IP3 channel receptors and, in consequence, blocks the IP3-induced calcium mobilization from sensitive stores[5].

Study design

We used 8 non-pregnant Sprague-Dawley female rats, weighting 180-200g. There were selected only animals being in diestrum, by vaginal smear examination.

The animals were kept in cages, with 8 hours of light/day, with permanent water supply and with a normal diet, established by the Nutrition and Epidemiology Departments.

They were killed by rapid decapitation, after being put to sleep with thiopental sodic 1g/kg. The trunks were sectioned and the two uterine horns from each animal were introduced in Krebs solution, with the following composition (mM): NaCl, 1.9 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$ and 5 glucose, oxygenated with a mixture of 95% O_2 and 5% CO_2 and thermostated at 37^0C.

For each animal two 3mm-length strips were cut from each uterine horn. For strips were, therefore, obtained from each rat and a total of 32 strips were used for the experiment.

All experiments were performed under the American University Laboratory Animal Care Committee Agreement.

The uterine strips were mounted vertically in a 5-ml organ bath and connected to a force transducer (ML T0201/RAD; ADInstruments, Colorado Springs, CO, USA) coupled to a Quad Bridge Amplifier (ADInstruments). Contractions were recorded using a PowerLab system and Chart 5 software (ADInstruments).

After 15 minutes of equilibration, the strips were washed with warm Krebs solution and, after 10 minutes, a control contraction to angiotensin II 10-7M (AG II), was induced. The contraction was quantified during 10 minutes.

The strips were washed 3 times, each 10 minutes and after 5 minutes xestospongine C (XeC) was introduced, thus its concentration in the organ bath was 10-7M. After another 5 minutes, AG II was readministered in the same concentration and the contraction obtained was compared to control.

The contractile response was analyzed by using 2 parameters:

a) area under the contractility curve.

b) frequency of myometrial contractions.

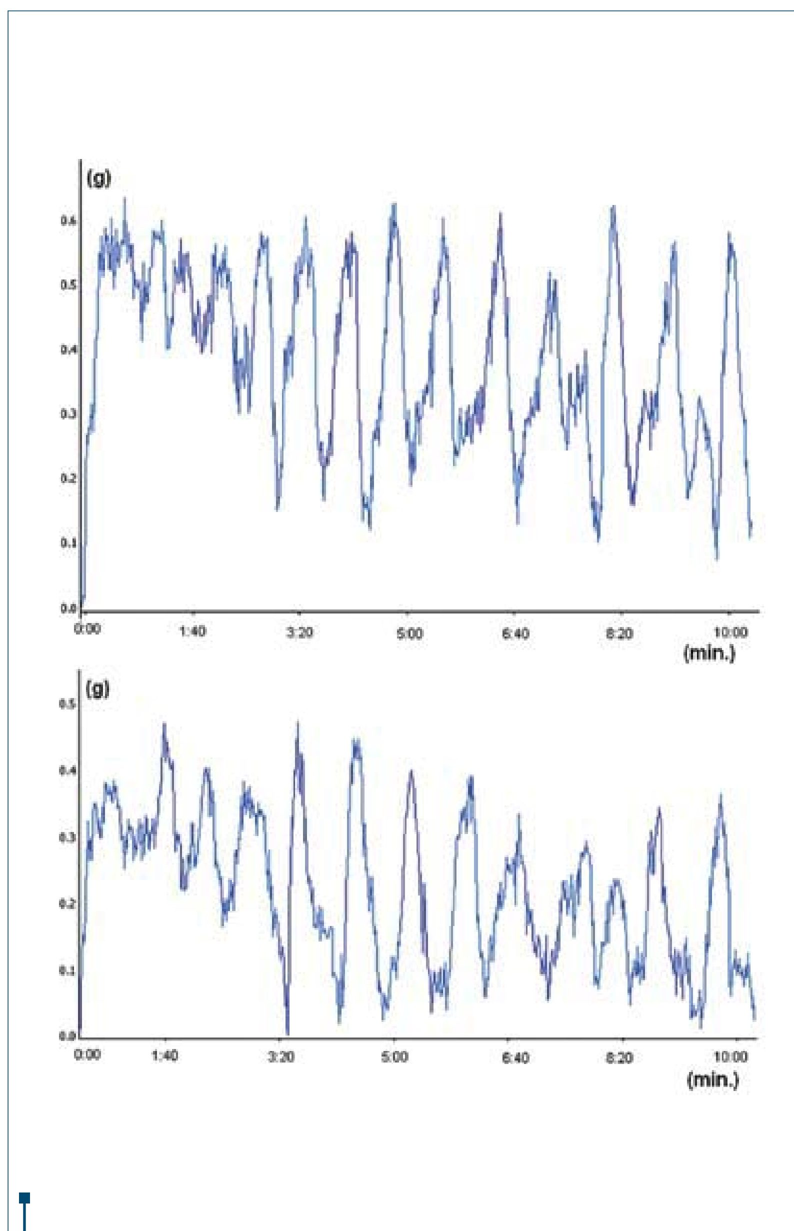

Figure 2. Details of the AG 10-7M-induced contraction before (on the top) and after Xe C 10-7M administration (on the bottom)

Statistical analysis

Two-way ANOVA was used; a P-value of <0.05 was considered statistically significant.

Chemicals and reagents

Experiments were performed with angiotensin II (Sigma-Aldrich) and xestospongin C (Sigma-Aldrich).

Results

Spontaneos activity of 3mm uterine slices was characterized by a mean area under the contractility curve of 98,87±47g.s and by a frequency of oscillations of 14±6/10min.

AG II 10-7M induced a strong contractile effect: area under the contractility curve increased to 331,7±43g.s and the frequency to 18±3/10min.

After Xe C 10-7M administration, AG II 10-7M induced a decreased contractile effect, when compared with AG II 10-7M alone: the area under the contractility curve decreased with 34,12±11% and the frequency of the myometrial contractions with 18,75±5% ($p < 0.05$) (Figures 1, 2).

It can be observed that Xe C 10-7M also decreased the amplitude of the spontaneous uterine contractions with 38,79±8%, without any impact on their frequency ($p < 0.05$) (Figure 1).

Disscusion and conclusions

Myometrium, as all types of smooth and skeletal muscle is completely dependent on intracellular concentration of calcium. This means that it is possible to evaluate the role of each component of intracellular calcium dynamics on myometrial-induced contraction, by blocking separately each way to increase the concentration of calcium in cytosol.

As shown in Figure 1, it seems that during AG II-induced contraction, IP3 was responsible for 34,12±11% of total calcium mobilized in cytosol.

This effect is stronger on amplitude of the contractions since the frequency is decreased only with 18,75±5%.

In the physiology of the automatic uterine activity IP3 has also a significant role on amplitude of the contractions (38,79±8%). But the frequency is not IP3-sensitive calcium stores dependent, prooving that IP3 is not implicated in spontaneous membranal depolarizing (Figure 1).

This is for the first time when Xe C is used to evaluate the implication of IP3-sensible intracellular calcium stores, during AG II-induced uterine contraction.

We conclude that it IP3-sensitive calcium stores seem to have a significant role in AG II-induced contraction. This implication is stronger on force than on frequency of oscillations. ■

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