Cyclic ADP-ribose/Ca^{2+} system in uterine smooth muscle cells

Abstract

Ryanodine (Ry)-sensitive calcium stores can be activated by cyclic adenosine diphosphate ribose (cADPR) and by Ca2+ through Ca2+-induced Ca2+-release (CICR), both intracellular signaling pathways being initiated by almost all oxytocics. ***Objective.*** *We investigated the role of Ry-sensitive calcium stores on spontaneous and angiotensin II (AGII)-induced uterine contraction by blocking Ry receptors with Ry in a rat model. From our knowledge, this is the first study on AGII effects on internal calcium mobilization, after cADPR pathway activation.* ***Methods.*** *The characteristics of myometrial contraction before and after Ry 10-6M administration were measured, using non pregnant female Sprague-Dawley rats (n=20) uterus.* ***Results.*** *Ry 10-6M did not influence spontaneous activity. Using Ry at 10-6M concentration, the amplitude and the frequency of AGII-induced contraction were showed to decrease with 11.62±4% and 9.78±3%, respectively.* ***Conclusions.*** *Our results suggest that one of the mechanisms through which angiotensin II induces an oxytocic effect is the mobilization of calcium from the calcium stores by activating the Ry-sensitive calcium channels.*

Keywords: *calcium stores, myometrium, angiotensin II, ryanodine, cADP ribose, rats*

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Introduction

Internal calcium is stored, almost exclusively, in endoplasmic and lysosomal vesicles[1]. The first one, belonging to the smooth reticulum, have two types of channels-receptors, through whom calcium can be mobilized, inositol triphosphate (IP3)-sensitive and ryanodine Ry)-sensitive calcium receptor-channels[2].

Ry-sensitive channels can be activated through two pathways: by calcium and by cyclic adenosine diphosphate ribose (cADPR). The first mechanism, called calcium-induced calcium-release (CICR)[3], is due to the calcium arised either by mobilization from IP3-sensitive stores (less important, from other internal stores types) or by entering through membranal L-type channels. The other pathway is dependent on generation of cADPR[4], due to the ADP-ribozil cyclase or cluster designation 38 activity on nicotinic amid adenine dinucleotid phosphate[5]. This enzyme is activated after the binding of an ocytocic on its specific G-protein coupled receptor[6].

It is important to mention that 50-60% of the endoplasmic vesicles have both types of calcium channels and only 20% of them possess Ry-sensitive calcium channels alone[7,8]. This means that cADPR or calcium, can mobilize 70-80% of the total endoplasmic calcium.

In obstetrics, angiotensin II (AGII) is used in a vasopressor test, for the prognosis of preeclamptic/eclamptic disorders. At the same time, the substance can induce a strong oxytocic effect and we already showed its impact on the total intracellular calcium dynamics inside myometrial cells[9] and, separately, on IP3-sensitive internal calcium stores[10].

The purpose of this study was to show that AGII induces an oxytocic effect through the mobilization of calcium from the calcium stores by activating the Ry-sensitive calcium channels.

Methods

Animal model

Twenty non-pregnant female Sprague-Dawley rats, weighting between 180-200 g, were used. We only selected animals in diestrum, by vaginal smear examination. Animals were housed in standard polycarbonate cages with free access to water and were maintained on a 8:16-h light:dark cycle in a climatised room. The animals were fed with a normal diet, established by the Nutrition and Epidemiology Departments.

All animals used in this study were maintained in a facility accredited by Helsinki Declaration and guidelines of the Ethics Committee of the International Association for the Study of Pain. All experiments were performed under the American University Laboratory Animal Care Committee Agreement.Also, animals received human care in accordance with the National Institutes of Health's Guide for Care and Use of Laboratory Animals.

They were killed by rapid decapitation, after being put to sleep with thiopental sodium 1 g/kg. The trunks were sectioned and the two uterine horns from each animal were introduced in Krebs-Henseleit solution having the following composition (mM): 127 sodium chloride, 1.9 potassium chloride, 1.2 potassium dihydrogen phosphate, 2.4 calcium chloride, 1.3 magnesium chloride, 26 sodium bicarbonate and 5 glucose (pH= 7.4), oxygenated with a mixture of 95% oxygen and 5% carbon dioxide and thermostated at 37oC (all reagents provided from Sigma-Aldrich, USA). Each uterine horn was cut in strips of 3 mm length.

Further, 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, USA). Contractions were recorded using a PowerLab system and Chart 5 software (ADInstruments, USA).

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After 10 min. of equilibration, the strips were washed with warm Krebs-Ringer solution and the spontaneous uterine activity was recorded during 10 minutes, this trace being used as control.

Two types of experiments were performed:

a) the impact of Ry 10-6M on spontaneous myometrial activity,

b) the role of Ry-sensitive calcium stores on AGII-induced uterine contraction.

Two hypothesis were verified: (I) the impact of Ry 10-6M on spontaneous myometrial activity and (II) the role of Ry-sensitive calcium stores on AGII-induced uterine contraction. Both reagents used in this study being from Sigma-Aldrich, (USA).

After recording the control, Ry was addeded, up to a 10-6M concentration in the organ bath. After 10 min. the characteristics of spontaneous oscillations were measured, as described below, the obtained data being compared with controls.

For the second part of the experiment, we selected strips with little or no spontaneous activity, for better cuantifying the Ry impact. After recording the control, AGII was introduced, up to a 10-7M concentration in the organ bath and the contractile effect was measured during 10 min. Than, the strips were washed two times, each 10 min. and after 10 minutes from the last wash, Ry was addeded, up to a 10-6M concentration in the organ bath. After another 10 min., AGII 10-7M was readministered and the contraction was compared with control. We performed 8 determinations for each experiment.

The contractile response was analyzed using 2 characteristics of spontaneous oscillations: (a) area under the contractility curve and (b) frequency of myometrial contractions.

Statistical analysis

Data were analyzed by two-way ANOVA. A $p<0.05$ level of significance was accepted.

Results

Spontaneous activity of 3 mm uterine slices was characterized by a mean area under the contractility curve (contractions amplitude) of 104.37±39 gs and by a frequency of oscillations of 14±6/10 min. It was observed that Ry did not reduce the spontaneous uterine activity.

In contrary, AG II 10-7M induced a strong contractile effect, and this was expressed by the area under the contractility curve which increased to 331.7±43 gs and frequency to 18±3/10 min.

Compared with the AGII-induced myometrial contraction, in the case of combination of both AGII and Ry 10-6M, the contractility curve and the frequency of the oscillations were decreased with 11.62±4% and 9.78±7%, respectively (Figure 1)

Discussion

Ry receptors are calcium channels, situated on smooth endoplasmic reticulum sensitive calcium stores, being opened by Ca2+, cADPR and Ry in nM concentrations. At higher concentrations (i.e. μM) Ry blocks these receptors-channels[11].

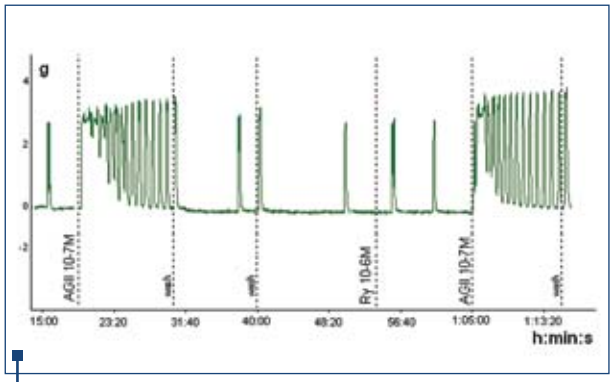

Figure 1. Effect of Ry 10^{-6}M on AGII 10^{-7}M induced myometrial contraction

Based on our results, we can say that, on AGII-induced myometrial contraction, Ry 10-6M showed to decrease the contractility curve with 11.62±4% and the frequency of the oscillations with 9.78±7%, respectively. It is, nevertheless, important to remember that 50-60% of the smooth endoplasmic vesicles have both types of calcium channels: IP3 and Ry-sensitive channels. Only 30% of Ry-sensitive calcium stores have the Ry receptors (20% from all smooth endoplasmic stores). This means that, , although Ry 10-6M blocks all the Ry-sensitive channels, a large amount of calcium, stored in smooth reticulum vesicles can be mobilized through IP3-sensitive channels. The 11.62±4% percentage reflects the role of smooth endoplasmic calcium stores, having only Ry-sensitive calcium channels (30% from all endoplasmic calcium stores). This means that the total impact of Ry-sensitive calcium stores on AGII-induced contraction is about 38.73%.

Conclusion

Our results suggest that one of the mechanisms through which angiotensin II induces an oxytocic effect is the mobilization of calcium from the calcium stores by activating the Ry-sensitive calcium channels. ■

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