Original Paper

Ocytocic Effect OF Angiotensin II and the Cyclic Ovarian Function – Study on Rat Myometrium

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ABSTRACT Myometrium is able to develop an intense spontaneous activity, without any external influences. However, endocrine environment can modulate, especially during pregnancy, the morphological and functional characteristics of the uterus. **OBJECTIVES:** The aim of this study is to quantify the impact of cyclic ovarian function on angiotensin II (AGII)-induced contraction of non-pregnant rat myometrium. **MATERIALS AND METHOD:** The experiments were performed on rat uterine strips, females being previously included in three separated groups, conform on their ovarian cycle phases: estrum, diestrum and proestrum. The AGII-induced ocytocic effect was compared with the spontaneous activity, by measuring 4 parameters: a)the mean and b)the maximal amplitude of the oscillations, c)their frequency (number/10min.) and d)the area under the contraction curve, during 10 minutes. **REZULTS:** Spontaneous activity was characterized: 1)in estrum by: a)1,86g, b)2,17g, c)15/10min, d)239g.s.; 2)in diestrum by: a)1,63g, b)1,78g, c)11/10min, d)165g.s.; 3)in proestrum by: a)1,47g, b)1,53g, c)3/10min., d)40g.s. AGII increased the contractility (quantified through area under the curve) with: 462% in estrum, 358% in diestrum and 1.202% în proestrum. **DISCUSSIONS:** Uterine spontaneous activity is maximal during estrum, this property being necessary for a rapid ascent of the sperm cells through female genital ways and for ovocytes capitation by the tubes. The minimal values were recorded in proestrum, the genital smooth muscle needing a rest period before its hyperactivity during estrum and, eventually, before a gestation. The diestrum was characterized by a medium intensity, together with a strong steadfastness of the uterine contractility. AGII-induced contractions followed very straightly the specific particularities of the spontaneous activity, excepting in proestrum, where its ocytocic effect was maximal. This last fact is the result of two phenomenon: 1)the automatic activity in proestrum is very weak and 2)during this period only the spontaneous depolarizing mechanisms are strongly inhibited, but not the force generating systems. **CONCLUSIONS:** Spontaneous and AGII-induced uterine contractility are strongly dependent on cyclic ovarian secretion. Despite in humans, the rat myometrium is far more sensible to the oscillatory hormonal plasma levels. It is no correlation between uterine contractility during different ovarian phases in human and rats, probable due to a completely different distribution and variability of the specific receptors.

KEY WORDS angiotensin II, myometrium, estrum, diestrum, proestrum

Introduction

Myometrium is able to develop spontaneous contractile activity, without any external stimulation. Its smooth muscle cells posses the capacity of "pacemaker", generating action potentials, which are rapidly spread through "gap" junctions in all uterus.

An important feature of the myometrium is its strong dependence on hormonal environment, cyclic ovarian activity and, especially, the pregnancy, being able to fundamentally modify the morphological and functional properties of the muscle.

These particularities are more important on rat, whose sexual cycle is composed by 4 periods: oestrum, proestrum, diestrum and metestrum, each one being characterized thorugh a specific vaginal smear [1].

Angiotensin II (AGII) consists in an eight amino acid peptide – Asp[1]-Arg-Val-Tyr-Ile-His-Pro-Phe[8] (angiotensin[1-8]). It is synthesized from plasmatic angiotensinogen (a 452 amino acid α_2-globulin in human [2]), produced mainly by the liver, but also in kidney, fat or in central nervous system [3,4].

Renine (released from the kidney) cleaves the poplipeptidic chain of angiotensinogen, resulting angiotensin I (or angiotensin[1-10]), which almost instantaneously is transformed in AG II, by angiotensin converting enzyme (ACE) activity [5].

Angiotensin II is one of the main endogen compounds responsible for blood pressure maintenance, due to several rapid effects (direct contractile action, stimulating of catecholamine release from sympathetic termination and adrenal medulla, inhibiting of catecholamine reuptake, increasing vascular responsiveness), slow effects (release of aldosteron from adrenal cortex and several renal actions) and due to vascular and

cardiac hypertrophy and remodeling effects [6]. This is the motif for the large utilization of several types of antihypertensive and antiedematous drugs, which the target is the renin-angiotensin system, such as inhibitors of ACE, antagonists of AG II receptors (AT1 blockers) or aldosteron antagonists.

In obstetrics and gynecology there are two directions of interest linked to angiotensin II: the possible association of a mild degree of hypertension on patients using oral contraceptives and the evaluation of vascular pressor responsiveness to AG II for diagnosis and prognostic of preeclampsia.

It is well known that estrogens increase plasmatic angiotensinogen levels, due to a hepatic synthesis stimulation (up to 8 times normal values [7}and despite the lack of strong proves of a link between oral contraceptives and higher blood pressure values, it was remarked an increased rate of essential [8] and pregnancy-induced hypertension in women using estro-progestative associations [9].

But the most important interest on AG II in obstetrics remains the pressor test at this peptide, in diagnosis and prognostic of preeclampsia/eclampsia diseases (pregnancy-induced or pregnancy-aggravated hypertension). Normally, pregnant women are characterized by low responsiveness to almost all vasoactive agents [10]. In these conditions, Gant and co-workers (1973) proved that an increased vascular sensitivity to AG II represents an accurate marker which precedes with several weeks the onset of pregnancy-induced hypertension [11]. They established that 90% of women who required more than 8ng/kg/min of AG II to induce a standardized pressor response between 28 and 32 weeks of pregnancy, will remain normotensive. Conversely, 90% of those to whom less than 8ng/kg/min of AG II provoke a pressor effect, between 28 and 32 weeks of pregnancy, will develop different degrees of pregnancy-induced hypertension. The possible aggravation of a chronic (pre-existing) hypertension in pregnant women, can, also, be prognosticated in the same way.

On rat myometrium AG II binds to two types of specific receptors: AT1 and AT2, both being membranal structures, coupled with G proteins. Secondary to the AT1 binding, phospolipase C (PLC) is activated, which will generate inositol 1,4,5 triphosphate (IP_3). This last second messenger will increase the intracellular calcium, with consecutive contraction [12]. AT2 have an unclear role, probable with antagonistic effect on AT1 [13].

It is also possible that AG II induces contraction on myometrium smooth muscle by binding other membrane sites than AT1 [14].

In humans, there is a dependency of the AT1 and AT2 density on hormonal status. In non-pregnant myometrium, in proliferative phase, AT2 are predominant [15]. In luteal phase, when uterus is preparing for an eventual gestation, the number of AT2 receptors decreases and the density of AT1 increases, favoring the hypertrophy and hyperplasia of the myometrium and the angiogenesis, phenomenon characteristic for pregnancy [16]. If gestation will appear, all mentioned processes will accentuate until term, when AT2 are almost absent [15].

Matherials and methods

We used non-pregnant Sprague-Dawley female rats, weighting 180-200 g. There were included in three groups, after the phase of their ovarian cycle: estrum, diestrum and proestrum, 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 $37^{\circ}C$.

From each uterine horn were cut 4 strips of 3 mm length.

All experiments were performed under the 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 10 minutes of equilibration, the strips were washed with warm Krebs solution and, for 10 minutes, the spontaneous activity was recorded. These was used as martor.

After this period, angiotensin II $10^{-7}M$ (AG II), was induced. The contraction was quantified during 10 minutes and compared with martor.

For each determination were performed 8 experiments.

The contractile response was analyzed by using 4 parameters:

a) maximal amplitude of the contractions;

b) mean amplitude of the contractions;

c) frequency of myometrial contractions (number/10min.).

d) area under the contractility curve (during 10min.).

We used angiotensin II (Sigma Aldrich (St. Louis, MO, USA);

Statistic analisys:

One-way ANOVA and two-way ANOVA were used.

$P < 0.05$ was considered statistically significant.

Results

Spontaneous uterine activity

Sponatenous activity, registered during different phases of rat ovarian cycle was characterized as mentioned in Table 1.

Table 1. The variability of different parameters of spontaneous activity of rat uterine strips (3/2/2mm), during rat ovarian cycle

	SPONTANEOUS ACTIVITY		
	ESTRUM	PROESTRUM	DIESTRUM
Mean amplitude (g)	1,86	1,47	1,63
Maximal amplitude (g)	2,17	1,52	1,78
Frequency (contractions/10 min.)	15	3	11
Area under the curve of contraction (g.s.)	239	40	165

Ocytocic effect of angiotensin ii on rat uterine strips, during diestrum phase of rat ovarian rat cycle

The effect begun with a false aspect of „tonic" type of contraction, due to the overlaping of several rapid oscilations (Fig. 1.).

AGII increased the area under the curve with 358%, until 755±149g.s.
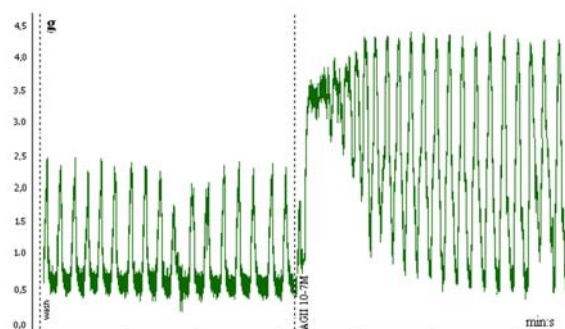
The maximal amplitude was 2,13±0,38g.
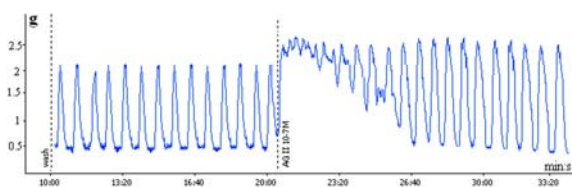
Figure 1. Angiotensin II $10^{-7}M$ effect on rat uterine strip, in diestrum (3/2/2mm).

Ocytocic effect of angiotensin ii on rat uterine strips, during estrum phase of rat ovarian rat cycle

AGII $10^{-7}M$ increased the area under the curve with 462%, the final value being 1343±257g.s. (Fig. 2.).

This effect was due especially to the strong impact on amplitude of the contractions: their mean being 3,83±0,47g and their maximum 3,96±0,59g.

It was maintened the false aspect „tonic-phasic" of the contractile curve, due to overlaping of several rapid oscilations, in the begining of the AGII-induced effect.

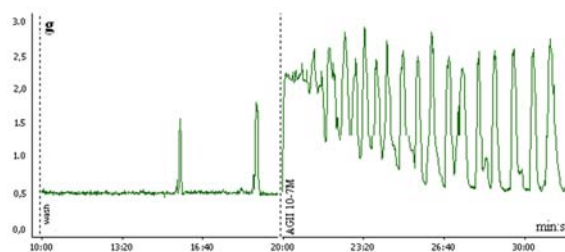**Figure 2. Angiotensin II $10^{-7}M$ effect on rat uterine strip, in estrum (3/2/2mm).**

Ocytocic effect of angiotensin ii on rat uterine strips, during proestrum phase of rat ovarian rat cycle

During proestrum, it was much easier to quantify the contractile effect of AGII $10^{-7}M$, due to its minimal spontaneous activity (Fig. 3.).

Maximal amplitude recorded was 2,38±0,519g.

The false „tonic-phasic" aspect or the grafic registration was also visible.

Area under the contraction curve was 481±94g.s., meaning an increase with 1202%.

Figure 3. Angiotensin II $10^{-7}M$ effect on rat uterine strip, in proestrum (3/2/2mm).

Discussions

As mentioned before, the myometrium is able to develop spontaneous activity, in the absence of

any external stimulus. However, the hormonal status can influence its structural and functional characteristics.

In humans, only pregnancy strongly modify the properties of the uterus, although it can be registered some modifications also during menstrual cycle.

Despite this, in rats, uterus is also very sensitive to ovarian cyclic function.

From our experiments, estrum is characterized by a maximal uterine contractility, almost 6 times over that recorded in proestrum (Tab. 1.). This aspect is logically, because during this phase the sperm cells must arrive quickly in tubes, and the ovocytes must be rapidly kept from the ovaries. In the same time, it can be observed the particular aspect of the oscillations, these presenting numerous irregularities, due to the powerful ionic currents implicated in depolarizing and force generation (Fig. 1).

Proestrum was the most "quiet" phase. Uterus needs a rest period before the hyperactive phase – estrum and, eventually, before a gestation. It is interesting the fact that in proestrum the frequency of contractions is very low, but the amplitude is not so weak. This means that only spontaneous depolarizing systems are strongly inhibited, but not the force generating mechanisms.

Estrum is characterized by a strong steadfastness of the uterine contractility. The amplitude of the spontaneous oscillations is medium, more than those in proestrum and less than those in estrum.

The ocytocic effect of angiotensin II (AGII) straightly follows the characteristics of myometrial contractile activity, specific to each of rat ovarian cycle phase.

It is maximal in estrum, minimal in proestrum and medium, but constant, in diestrum.

However, in proestrum, AGII increased the rat uterine contractility with 1.202%, This last fact is the result of two phenomenon: 1)the spontaneous activity in proestrum is very poor, so even a weak ocytocic effect of AGII will appear very strong and 2)during this phase only the spontaneous depolarizing mechanisms are strongly inhibited, but not the force generating systems.

There are important differences between the dependence of AGII-induced uterine contraction on hormonal status in humans versus in rats.

As mentioned before, in humans, significant influences on uterus appear only during pregnancy.

In rats, there are important modifications of myometrial contractility also linked to the rat ovarian cycle.

Moreover, in humans, the biggest density of AT1 receptors, responsible for angiotensin II-induced ocytocic effect, is recorded during late luteal phase, corresponding with the early diestrum phase in rats. Or, the maximal effect on rat uterus was registered in estrum, corresponding with mid-cycle in humans – periovulatory period.

In rats, the lowest contractility (theoretically due to a maximal number of AT2 receptors and a minimal number of AT1 receptors) was recorded in proestrum, corresponding with late proliferative phase in humans, where AT2 are decreasing and AT1 are increasing.

Conclusions:

Spontaneous and AGII-induced uterine contractility are strongly dependent on cyclic ovarian secretion.

The ocytocic effect of AGII straightly follows the evolution of automatic oscillations, excepting proestrum, where spontaneous activity is very weak, but AGII effect is significant.

Despite in humans, where only during pregnancy it can be observed significant uterine modifications, in rats, the myometrium is very sensitive to cyclic ovarian function.

Also, it is no correlation between different cyclic ovarian phases in human and rats, probable due to a completely different distribution and variability of the specific receptors, linked to the hormonal plasma levels.

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