The purpose of this study was to evaluate the effect of β<sub>3</sub>-adrenoreceptor agonist, CL 316243 on human non-pregnant uterine contractility. The activity of myometrium strips was recorded by means of force transducers with digital output. Quantification of the response of myometrium strips was done by calculation of the area under the curve (AUC), as well as the amplitude and frequency of contractions. CL 316243 in a concentration-dependent manner (10<sup>-10</sup>-10<sup>-4</sup> mol/L) decreased the AUC value (logIC<sub>50</sub> -8.088±0.29; n=16). Decreased mean frequency of contractions and nearly 30% inhibition of spontaneous contractile activity were also observed. The inhibition of contractions by CL 316243 was not changed by either butoxamine (selective β<sub>2</sub>-adrenoreceptor antagonist) or propranolol (β<sub>1</sub>- and β<sub>2</sub>-adrenoreceptor antagonist), and was partly antagonized by bupranolol (nonselective β-adrenoreceptor antagonist), each antagonist at 10<sup>-6</sup> mol/L. In conclusion: CL 316243 causes inhibition of spontaneous contractile activity of human non-pregnant myometrium. Our findings also indicate that CL 316243 attenuates the contractile activity of human non-pregnant myometrium by the β<sub>3</sub>-adrenoreceptors activation.

**Key words:** CL 316243, non-pregnant myometrium, contractile activity, human

**INTRODUCTION**

β<sub>3</sub>-Adrenoreceptors have been identified in a variety of tissues from humans and animals: white and brown adipose tissues, skeletal muscles, heart, gastrointestinal smooth muscles, respiratory tract or urogenital system (1).
β3-Adrenoreceptors have also been described in the uterus, where similarly to β2-adrenoreceptors, they play an important functional role in mediating relaxation of the myometrium (2). The presence of functional β3-adrenoreceptors has been recently demonstrated in human near-term (3) and non-pregnant myometrium (4).

Myometrial contractility is controlled by myogenic, neurogenic and hormonal mechanisms (5). In human myometrium, β3-adrenoreceptors are coupled to Gi protein and stimulate the adenylyl cyclase/cAMP pathway (6). β3-Adrenoreceptors agonists (e.g., BRL 37344, SR 59119A) are able to inhibit spontaneous contractions of human pregnant myometrium (3, 7). Recently, it has been published that β3-adrenoreceptors are the predominant subtype in human pregnant and non-pregnant myometrium (4).

According to our knowledge, no studies were published about the influence of CL 316243 on the human uterus. The sole report indicating the relaxing effect of CL 316243 on the myometrium was based on strips obtained from non-pregnant rats. (8). Thus, taking into account the species variability of β3-adrenoreceptor pharmacology (9), the purpose of our study was to evaluate the effect of CL 316243 on human non-pregnant uterine contractile activity.

MATERIALS AND METHOD

Human uterine tissues were collected from 27 non-pregnant, premenopausal women (aged 41-52 years, median 48) undergoing hysterectomy for benign gynecological disorders (dysfunctional bleeding) and cervical pre-malignancy (intraepithelial neoplasia) and being operated upon in the follicular phase of the menstrual cycle. All patients gave informed consent to the study which had been approved by the Ethics Committee of the Medical University of Bialystok. Myometrial samples were excised transversely from the fundus of the uterus, placed in ice-cold Tyrode's solution, and immediately transferred to the laboratory where they were processed as previously described (10). Briefly, 4-8 strips, 6-7 mm in length and 2x2 mm in cross-sectional area were obtained under a dissecting microscope.

The strips were then mounted in an organ bath containing 20 ml of Tyrode's solution at 37°C, pH 7.4, and bubbled with carbogen (95% O2 + 5% CO2). Tyrode's solution was composed of (mmol/L): NaCl 139.6; KCl 2.68; MgCl2 1.05; NaH2PO4 1.33; CaCl2 1.80; NaHCO3 25.0 and glucose 5.55. The strips were left in the organ bath for equilibration period of 1-2 hours. During that period, the passive tension was adjusted to 2 mN. After equilibration, regular phasic contractions were achieved.

The activity of the myometrium was recorded under isometric conditions by means of force transducers with digital output. CL 316243 was added cumulatively to the organ chambers (bath concentrations in the range 10-10 mol/L - 10-4 mol/L) at 15-minute intervals and the effects were recorded. The level of spontaneous contractile activity before the addition of CL 316243 was treated as a control level. The responses were quantified by calculating the area under the curve (AUC), the amplitude and frequency of the contractions. The AUC was measured from basal tension over a 10-minute period before the next addition of CL 316243.

In experiments with antagonists, the tissue responses were evaluated by comparing with those obtained in the presence of an antagonist, before the administration of the first concentration of CL 316243. Butoxamine - β2-adrenoreceptor antagonist, propranolol - β1- and β2- adrenoreceptor
antagonist and bupranolol - nonselective adrenoreceptor antagonist (each at concentration $10^{-6}$ mol/L), were added to the organ bath 20 minutes before the onset of the CL 316243 administration.

**Drugs and solutions**

CL 316243 was obtained from Sigma-Aldrich (St. Louis, MO, USA). A stock solution ($10^{-2}$ mol/L) of CL 316243 was prepared using redistilled water. Series of dilutions were prepared with redistilled water on the day of experiment and were maintained at room temperature for the duration of the experiment. Propranolol and butoxamine were obtained from Sigma-Aldrich. Bupranolol was a gift from Schwarz Pharma AG (Munchenstein, Switzerland). Stock solutions ($10^{-3}$ mol/L) off all three antagonists were made with redistilled water. All substances were added directly to the organ bath containing Tyrode's physiologic salt solution made daily.

**Statistical analysis**

The mechanical response of myometrial strips (AUC) was measured by the calculation of the integral of the appropriate section of the curve with the DASYLab software unit (Data Acquisition System Laboratory), version 9.0. Concentration-response curves were fitted to the logistic equation by computer analysis, using nonlinear regression (PRISM 3.0, Graph Pad Software INC., San Diego, Ca, USA). The concentration of agents that resulted in half-maximal inhibitory effect was expressed as $-\log (IC_{50})$.

All data were analyzed statistically with PRISM 3 using Student's t-test, two-way or one-way ANOVA, where appropriate. The statistical significance was considered when the probability value was $p<0.05$. Throughout the paper, all results are expressed as mean ± S.E.M., and n denotes the number of tissues from different patients.

**RESULTS**

All experiments were performed on myometrial strips exhibiting regular spontaneous contractile activity after equilibration (Fig. 1A). The mean frequency of contraction was 3.382±0.22 per 10 minutes, and the mean amplitude was 3.233±0.36 mN (n=36).

**The effects of CL 316243 on spontaneous myometrial contractions**

The cumulative addition of CL 316243 resulted in a concentration-dependent decrease of the myometrial strips activity (Fig. 1B) seen as a significant decrease of the AUC value (Fig. 2). The IC$_{50}$ value was -8.088±0.29 (n=16) and the mean maximal inhibition was 27.74±1.85 % (Table. 1). In parallel with this effect a significant decrease in the mean frequency of contractions has been observed. However, CL 316243 did not influence significantly the mean amplitude of contractions (Fig. 3).

**The influence of β-adrenoreceptors antagonists on myometrial contractile activity**

At first, we checked the influence of the antagonists: butoxamine, propranolol and bupranolol (each at $10^{-6}$ mol/L), on spontaneous contractile activity of
myometrial strips. The incubation of myometrial strips with propranolol, or bupranolol resulted in a significant decrease in the AUC and the amplitude of spontaneous contractions (Fig. 4). For this reason, in the group of experiments with antagonists, the tissues responses to CL 316243 were evaluated by comparing with those obtained in the presence of the antagonist, before the administration of the first concentration of CL 316243.

**The effects of ß-adrenoreceptors antagonists on CL 316243-induced relaxation of spontaneous myometrial contractile activity**

**Butoxamine**

A plot of the AUC as the function of CL 316243 concentration indicated that pretreatment with 10⁻⁶ mol/L butoxamine did not counteract the CL 316243-induced relaxation of the spontaneous contractions of the myometrial strips. A slight shift of the concentration-response curve was statistically insignificant (Fig. 5, and Table. 1).
Admittedly, butoxamine augmented the decrease of the mean amplitude of contractions induced by CL 316243, but this effect was significant only for the highest concentrations of the agonist. The influence of butoxamine on the decrease in the mean frequency induced by CL 316243 was also statistically insignificant (Fig. 6).

Propranolol

There was no significant variation in the mean value of the maximal inhibition for CL 316243 in the absence (27.74±1.85%) and presence (30.09±3.38%) of propranolol. Preincubation of myometrial strips with propranolol did not alter the

Table 1. Log IC$_{50}$ and the mean maximal inhibition (%) of CL 316243 in the absence and presence of β-adrenoreceptors antagonists.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>IC$_{50}$</th>
<th>Mean maximal inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (n=16)</td>
<td>-8,088±0.29</td>
<td>27.74±1.85</td>
</tr>
<tr>
<td>Butoxamine (n=5)</td>
<td>-7,505±0.18</td>
<td>29.09±3.38</td>
</tr>
<tr>
<td>Propranolol (n=5)</td>
<td>-8,885±0.57</td>
<td>30.09±3.38</td>
</tr>
<tr>
<td>Bupranolol (n=10)</td>
<td>-5,869±0.67 *</td>
<td>16.27±3.59 *</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM
(*p< 0.05) versus effects of CL 316243 on myometrial strips that were not preincubated with bupranolol.
Fig. 3. The cumulative effect of CL 316243 on the amplitude and frequency of contractions of non-pregnant human myometrial strips. The amplitude or frequency of the spontaneous contractile activity were treated as a control values. Asterisks indicate values significantly different from control (p<0.05).

Fig. 4. The influence of antagonists of the β-adrenoreceptors (butoxamine, propranolol and bupranolol, each at concentration $10^{-6}$ mol/L) on spontaneous contractions of myometrial strips. Asterisks indicate values significantly different from control (p<0.05).
Fig. 5. The effects of butoxamine (n=5), propranolol (n=5) and bupranolol (n=10) on CL 316243-induced relaxation of the myometrial spontaneous contractile activity (AUC). Spontaneous contractions of the myometrial strips before the addition of CL 316243 were treated as a control. Each point represents mean±S.E.M.

Fig. 6. The influence of butoxamine (10⁻⁶ mol/L) on changes in amplitude and frequency induced by CL 316243. Respectively, the amplitude and frequency of spontaneous contractions before the addition of CL 316243 were treated as a control. Asterisks indicate values significantly different from control (p<0.05).
concentration-response curve for CL 316243. The mean values of IC$_{50}$ and maximal inhibition did not differ significantly before and after the tissue incubation with propranolol (Fig. 5, and Table 1).

The presence of propranolol in the organ bath did not modify the mean amplitude of contractions, but significantly (in the concentration range 10^-8 mol/L to 10^-5 mol/L) deepened the decrease of the mean frequency induced by rising concentration of CL 316243 (Fig. 7).

**Bupranolol**

Preincubation of myometrial strips with bupranolol resulted in a significant rightward shift of the concentration-response curve for CL 316243 (Fig. 5). There was a significant difference between the IC$_{50}$ value in the absence (-8.088±0.29) and presence (-5.869±0.67) of bupranolol. Furthermore, there was a significant decrease of the maximal inhibition in the absence and presence of the antagonist, 27.74±1.85 and 16.27±3.59, respectively (Table 1). The presence of bupranolol in the organ bath did not significantly affect the CL

![Fig. 7. The influence of propranolol (10^-4 mol/L) on changes in amplitude and frequency induced by CL 316243. Respectively, the amplitude and frequency of spontaneous contractions before the addition of CL 316243 were treated as a control. Asterisks indicate values significantly different from control (p<0.05).](image-url)
316243-induced changes of the mean amplitude and frequency of contractions (Fig. 8).

DISCUSSION

Recently, increased interest in the role of β₃-adrenoreceptors in the regulation of contractile activity of smooth muscles, including myometrium, has been observed (2, 11, 15).

CL 316243 is a selective and potent β₃-adrenoreceptor agonist with thermogenic properties (16). It also mediates lipolysis in adipose tissue (17). Several studies have demonstrated the species variability of β₃-adrenoreceptor pharmacology, as well as the heterogeneous responsiveness to β₃-adrenoreceptor agonists of tissues in a given species (9). It has also been reported that CL 316243 relaxes the human detrusor (19) and human ureteral smooth muscles (20). However, according to our knowledge, its ability to relax human uterus has not been reported.
Our data indicate that CL 316243 attenuates spontaneous contractile activity of the human non-pregnant myometrium in a concentration-dependent manner. A comparison of our results with the effects caused by SR 59119A (4) suggests that the maximal inhibition which CL 316243 brings about is smaller than that of SR 59119A. The difference in the potencies of CL 316243 (the present data) and SR 59119A may result from the fact that we used the AUC values, whilst in the paper of Rouget et al. (4), the amplitude was used to construct the concentration-response curve. Our data indicate that CL 316243, first of all, influences the frequency of the contractions of myometrial strips while the changes of the amplitude were not seen before the highest concentration of CL 316243 was added to the organ bath. This observation is in accordance with the opinion (21) that the area under the curve is a parameter that more precisely reflects the activity of smooth muscles characterized by phasic contractions.

In the next step of our studies, we performed an evaluation of the β-adrenoreceptor subtype activated by CL 316243. Dennedy et al. (22) used butoxamine, propranolol, and bupranolol, all at concentration 10^{-6} mol/L, to block β-adrenoreceptors in the human pregnant myometrium. In our experiments, we used the same concentration of the β-adrenoreceptors antagonists. Because the incubation with bupranolol or propranolol significantly decreased both the AUC value and the mean amplitude of contractions, in this group of experiments, the contractile activity recorded in the presence of antagonists was taken as a control to exclude the undesired effects of antagonists.

The CL 316243-induced attenuation of the spontaneous contractile activity was resistant to blockade with the β_1- and β_2-adrenoreceptors antagonist, propranolol, and β_2-adrenoreceptors antagonist, butoxamine. Statistically insignificant shifts of the concentration-response curves for CL 316243 observed in the presence of propranolol or butoxamine (leftward or rightward, respectively) may be, at least in part, a result of differences in the β_3-adrenoreceptors density. Sakakibara et al. have observed a correlation between the diversity of inhibitory responses to β_2-adrenoreceptors agonists and the differences of the β_2-adrenoreceptors density in smooth muscles of human pregnant uteri at term (23). On the other hand, the leftward shift of the concentration-response curve observed in the presence of propranolol may result from the ability of propranolol to block α_1-adrenoreceptors (24). Further studies are necessary to elucidate this problem.

The nonselective β_1-, β_2- and β_3-adrenoreceptors antagonist, bupranolol caused a statistically significant rightward shift of the concentration-response curve for CL 316243.

The results show that CL 316243 relaxes the spontaneous contractile activity of the non-pregnant human myometrium via β_3-adrenoreceptors activation.

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