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Differences in the effects of β_2- and β_3-adrenoceptor agonists on spontaneous contractions of human nonpregnant myometrium

Odmienny wpływ agonistów receptorów β_2- i β_3-adrenergicznych na spontaniczne skurcze myometrium kobiet nieciężarnych

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Abstract

Objective: *This study aimed to compare the relaxant properties of BRL 37344 with β_2-adrenoceptors agonist ritodrine on the contractility of human nonpregnant myometrium.*

Material and methods: *The activity of myometrial strips mounted in an organ bath was recorded under isometric conditions using force transducers with digital output. Contractility before and after cumulative additions of both uterorelaxants and with preincubation with β-adrenoceptor antagonists bupranolol, propranolol, and butoxamine were studied.*

Results: *Both BRL 37344 (10^{-10} – 10^{-4} mol/L) and ritodrine (10^{-10} – 10^{-5} mol/L) decreased the area under curve, or AUC, value ($logIC_{50}$ -6.45 ± 0.18 and -8.71 ± 0.35, respectively), and the degree of inhibition of spontaneous contractile activity was similar (< 30%). However, BRL 37344 decreased the mean frequency of contractions, whereas ritodrine decreased the mean amplitude of contractions. The inhibition of contractions by BRL 37344 was partially antagonized by bupranolol and propranolol, but not with butoxamine. The inhibition by ritodrine was counteracted by all these antagonists.*

Conclusions: *The effects of BRL37344 and ritodrine on human nonpregnant myometrium are quantitatively similar in respect to the inhibition of spontaneous contractility, yet are also distinct due to their substantially different influences on contraction parameters. Our data indicate that β_3-adrenoceptor activation is not the sole effect of BRL 37344 on this tissue.*

Key words: **adrenergic agonists** / **BRL 37344** / **human** / **myometrium** / **ritodrine** /

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Streszczenie

Cel pracy: *Niniejsze badanie zaprojektowano celem oceny rozkurczowego działania BRL 37344 w porównaniu do ritodryny (referencyjnego agonisty receptorów β_2-adrenergicznych) na spontaniczną czynność skurczową ludzkiego nieciężarnego myometrium.*

Materiał i metody: *Skurcz izolowanych skrawków mięśni gładkich rejestrowano w warunkach izometrycznych przy użyciu cyfrowych przetworników siły. Ocenie poddawano zmiany kurczliwości skrawków podczas kumulacyjnego zwiększania stężeń agonistów bez obecności oraz w obecności antagonistów receptorów β adrenergicznych: bupranololu, propranololu oraz butoksaminy.*

Wyniki: *Inkubacja skrawków myometrium z BRL 37344 (10^{-10} – 10^{-4} mol/L) oraz ritodryną (10^{-10} – 10^{-5} mol/L) skutkowała porównywalnym (<30%) osłabieniem ich kurczliwości przejawiającym się spadkiem wartości pola powierzchni pod krzywą, czyli AUC (odpowiednio, $logIC_{50}$ -6,45 ± 0,18 oraz -8,71 ± 0,35). W odróżnieniu od ritodryny, której efekt działania polegał na obniżaniu średniej amplitudy skurczu, inkubacja z BRL 37344 zmniejszała średnią częstotliwość skurczu. Działanie rozkurczowe BRL 37344 było częściowo hamowane przez propranolol oraz bupranolol, podczas gdy wpływ ritodryny był znoszony przez wszystkich zastosowanych antagonistów.*

Wnioski: *Porównywalne ilościowo działanie rozkurczowe BRL 37344 oraz ritodryny na spontaniczną czynność skurczową ludzkiego myometrium jest wynikiem odmiennego wpływu tych agonistów na poszczególne parametry skurczowe. Nasze dane wskazują, iż aktywacja β3-adrenoceptorów nie jest jedynym kierunkiem działania BRL 37344 na tę tkankę.*

Słowa kluczowe: **agoniści receptorów adrenergicznych** / **BRL 37344** / **człowiek** / / ***myometrium*** / **ritodryna** /

Introduction

Both receptors subclasses, β_2- and β_3-adrenoceptors have been shown to mediate the contractile response of the human uterus. The regulation of the β-adrenoceptors in humans may vary due to numerous pharmacologic, physiologic, and hormonal influences, and the impact of the phases of the menstrual cycle, pregnancy, or menopause [1]. Furthermore, the β_3-adrenoceptors have been characterized and their functionality shown in pregnant human myometrium [2, 3]. The expression of β_3-adrenoceptors increases in the near-term myometrium [1]. Two populations of binding sites corresponding to β_2- and β_3-adrenoceptors have been identified, and the predominance of the β_3-adrenoceptors subtype in the nonpregnant and pregnant human myometrium were also confirmed [4].

β_2-Adrenoceptor agonists, such as ritodrine, are well-known drugs used for the management of preterm labor. However, maternal and fetal cardiovascular side effects restrict their limitations in clinical practice [5]. According to our previous study, a β_3-adrenoceptors agonist, CL 316243, efficiently inhibits spontaneous contractions of human nonpregnant myometrium [6]. This finding is parallel to the Rouget *et al.* study, in which a β_3-adrenoceptors agonist SR 59119A was shown to diminish spontaneous rhythmic contractile activity in this tissue [4]. The influence on the contractile activity of another interesting β_3-adrenoceptors agonist, BRL 37344, has been studied in the pregnant human myometrium [3, 7]. Some reports, indicating a marked relaxing effect of BRL 37344 on the nonpregnant uterine smooth muscle, were based on experiments on rats and pigs [8, 9]. Surprisingly, there is virtually no information available on the agent's effects on human nonpregnant uterus.

Therefore, taking into account the species variability of β_3-adrenoceptor pharmacology investigated [10], the purpose of the present study was to compare the ability of BRL 37344 to relax spontaneous contractility of the human nonpregnant myometrium with that of ritodrine.

Material and methods

Human myometrial tissue

Human uterine tissues were collected from 20 nonpregnant, premenopausal women (aged 42-50 years, median 48 years) undergoing hysterectomy for benign gynecological disorders, such as dysfunctional bleedings and cervical pre-malignancy, and being operated upon in the follicular phase of their menstrual cycles. All patients gave informed consent to the study which had been approved by the Ethics Committee of the Medical University of Białystok (approval number R-I – 003/307/2005).

Myometrial samples were excised transversely from the uterine fundus, placed in ice-cold Tyrode's solution, and immediately transferred to the laboratory where they were processed as previously described [6]. 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 ºC, pH 7.4, and bubbled with carbogen (95% O_2 + 5% CO_2). Tyrode's solution consisted of (mmol/L): NaCL 139.6; KCl 2.68; $MgCl_2$ 1.05; NaH_2PO_4 1.33; $CaCl_2$ 1.80; $NaHCO_3$ 25.0; and glucose 5.55. The strips were left in the organ bath for equilibration period of 1-2 hours, during which time 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. BRL 37344 or ritodrine was added cumulatively to the organ chambers (bath concentrations in the range 10^{-10} mol/L – 10^{-4} mol/L and 10^{-10} mol/L – 10^{-5} mol/L, respectively) at 15-minute intervals, and the effects were recorded. The level of spontaneous contractile activity before the addition of agonist was regarded as the control level. The responses were quantified by: calculating the area under the curve (AUC) of active contraction [11], the amplitude, frequency of the contractions, and the basal tension. The AUC was measured from basal tension over a 10-minute period before a further addition of β-adrenoceptor agonist.

Effect of preincubation with β-adrenoceptor antagonists

The tissue responses were evaluated by comparing them with those obtained with the antagonist, before the administration of the first concentration of BRL 37344 or ritodrine. Butoxamine (β_2-adrenoceptor antagonist), propranolol (β_1- and β_2-adrenoceptor antagonist), and bupranolol (nonselective adrenoceptor antagonist), each at concentration of 10^{-6} mol/L, were added to the organ bath 20 minutes before the agonists administration.

Drugs and solutions

BRL 37344 and ritodrine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of BRL 37344 (10^{-2} mol/L) and ritodrine (10^{-3} mol/L) were prepared daily using bidistilled water. Series of dilutions were prepared with bidistilled water on the day of experiment and were maintained at room temperature for the duration of the experiment. Propranolol and butoxamine were also from Sigma-Aldrich. Bupranolol was obtained from Schwarz Pharma AG (Münchenstein, Switzerland). Stock solutions (10^{-3} mol/L) of the three antagonists were made with bidistilled water. All substances were added directly to the organ bath containing Tyrode's solution also made on daily basis.

Measurement of contraction parameters

The AUC parameter, representing mechanical response of myometrial strips, was measured by calculating the integral of the appropriate section of the curve [12] with the DASYLab software unit (version 9.0; Laboratory Data Acquisition System, SuperLogics, Waltham, MA, USA). 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 concentrations of agents that resulted in half-maximal inhibitory effect were expressed as –log (IC_{50}).

Statistical analysis

All results are expressed as means ± SEM and "N" denotes the number of tissues from different patients. Data were analyzed statistically with PRISM 3 using Student's *t*-test and a two- or one-way ANOVA, where appropriate. The statistical significance was considered when the probability value (*P*) was <0.05.

Results

All experiments were performed on myometrial strips exhibiting regular spontaneous contractile activity after equilibration. The mean frequency of contractions was 3.46 ± 0.36 per 10-minute interval, the mean amplitude was 3.52 ± 0.29 mN, and the mean basal tension was 1.69 ± 0.31 mN (N = 52).

Effect of BRL 37344 and ritodrine on myometrial strips activity

The cumulative addition of BRL 37344 or ritodrine resulted in a concentration-dependent decrease of the myometrial strips activity seen as a significant decrease in the AUC value (Figures 1 and 2). The difference of the IC_{50} values for the two agonists was statistically significant ($P < 0.05$; Table I).

For BRL 37344 alone (N = 5), the IC_{50} value was -6.45 ± 0.18 and the mean maximal inhibition was 24.94 ± 1.85% (Table I). At the same time, a significant decrease of both the mean frequency of contractions and the basal tension was observed. However, BRL 37344 did not significantly influence the mean amplitude of contractions. (Figure 3).

For ritodrine alone (N = 13), the IC_{50} value and the mean maximal inhibition were -8.71 ± 0.35 and 29.07 ± 2.24%, respectively. (Table I).

Ritodrine caused a significant decrease of the mean amplitude of contractions and the basal tension. In contrast to BRL 37344, ritodrine did not influence the mean frequency of contractions (Figure 3). In addition, preincubation of myometrial strips with ritodrine caused a pronounced increase in the duration of contractions. (Figure 1).

As we have previously shown [6], preincubation of nonpregnant uterine strips with propranolol or bupranolol significantly decreases the AUC values and the amplitude of myometrial contractions.

Table I. Log IC_{50} (half-maximal inhibitory effect) and the maximal inhibition (%) of BRL 37344 and ritodrine in the absence and presence of β-adrenoceptors antagonists. Data are expressed as mean ±SEM.

	IC_{50}		Maximal inhibition (%)	
	BRL 37344	**Ritodrine**	**BRL 37344**	**Ritodrine**
None	-6.45 ± 0.18 (N = 5)	-8.71 ± 0.35 [a] (N = 13)	75.06 ± 1.47 (N = 5)	70.93 ± 2.24 (N = 13)
Butoxamine	-6.19 ± 0.20 (N = 7)	-	68.23 ± 3.38 (N = 7)	-
Propranolol	-7.44 ± 0.34 [b] (N= 5)	-	61.31 ± 3.15 [c] (N = 5)	-
Bupranolol	-7.34 ± 0.65 (N = 6)	-7.59 ± 0.49 (N = 6)	83.97 ± 2.84 [d] (N = 6)	76.99 ± 4.19 (N = 6)

Significant differences ($P < 0.05$) were noted for:
– IC_{50}: BRL 37344 in myometrial strips that were not preincubated (-6.45 ± 0.18) versus ritodrine [a] and BRL 37344 in presence of propranolol [b];
– Maximal inhibition: BRL 37344 in myometrial strips that were not preincubated (75.06 ± 1.47) versus BRL 37344 in presence of propranolol [c] or bupranolol [d].

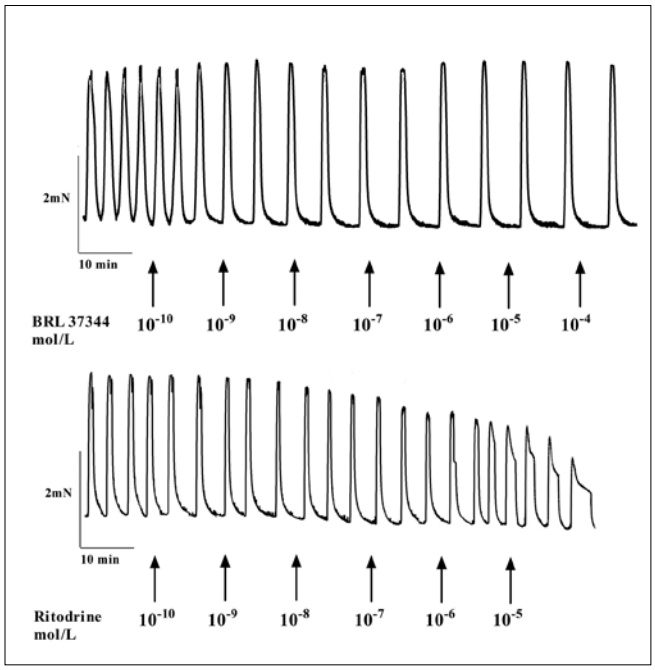

Figure 1. A representative recording of spontaneous contractile activity of the human nonpregnant myometrial strips and the effect of cumulatively administered: A – BRL 37344 (10^{-10} – 10^{-4} mol/L), and B – ritodrine (10^{-10} – 10^{-5} mol/L).

Therefore, in the experiments with the antagonists, the muscular responses to agonists were evaluated by comparing them with those obtained in the presence of the antagonist but before the administration of the first concentration with agonist.

Effect of butoxamine on the relaxation response to BRL 37344 and ritodrine

The AUC as a function of BRL 37344 concentration indicated that pretreatment with 10^{-6} mol/L butoxamine did not counteract the BRL 37344-induced relaxation of the spontaneous contractions of the myometrial strips. A slight shift of the concentration-response curve was not statistically significant. (Figure 2A and Table I). The BRL 37344-induced changes of the mean frequency and basal tension were not significantly influenced by butoxamine. (Figures 3C and E).

Preincubation of myometrial strips with butoxamine resulted in a significant shift of the concentration-response curve to the right for ritodrine. (Figure 2B and Table I). There were no changes of the mean values of amplitude and frequency after the incubation of the myometrium strips with butoxamine. However, pretreatment with butoxamine abolished the effect of ritodrine on the basal tension. (Figure 3F).

Effect of propranolol on the relaxation response to BRL 37344 and ritodrine

The addition of propranolol to the organ bath before the first administration of BRL 37344 caused a statistically significant shift to the left of the concentration-effect curve for BRL 37344. (Figure 2A and Table I). There was no influence of propranolol on the decrease of the mean frequency of contractions. On the other hand, preincubation with propranolol abolished the decrease of the basal tension caused by BRL 37344. This effect was statistically significant for the highest concentration of the agonist. (Figure 3F).

Preincubation of myometrial strips with propranolol resulted in a significant rightward shift of the concentration-response curve for ritodrine. The range of ritodrine concentrations used in our experiments (10^{-10} – 10^{-5} mol/L) did not allow to fit the AUC values to the logistic equation. (Figure 2B).

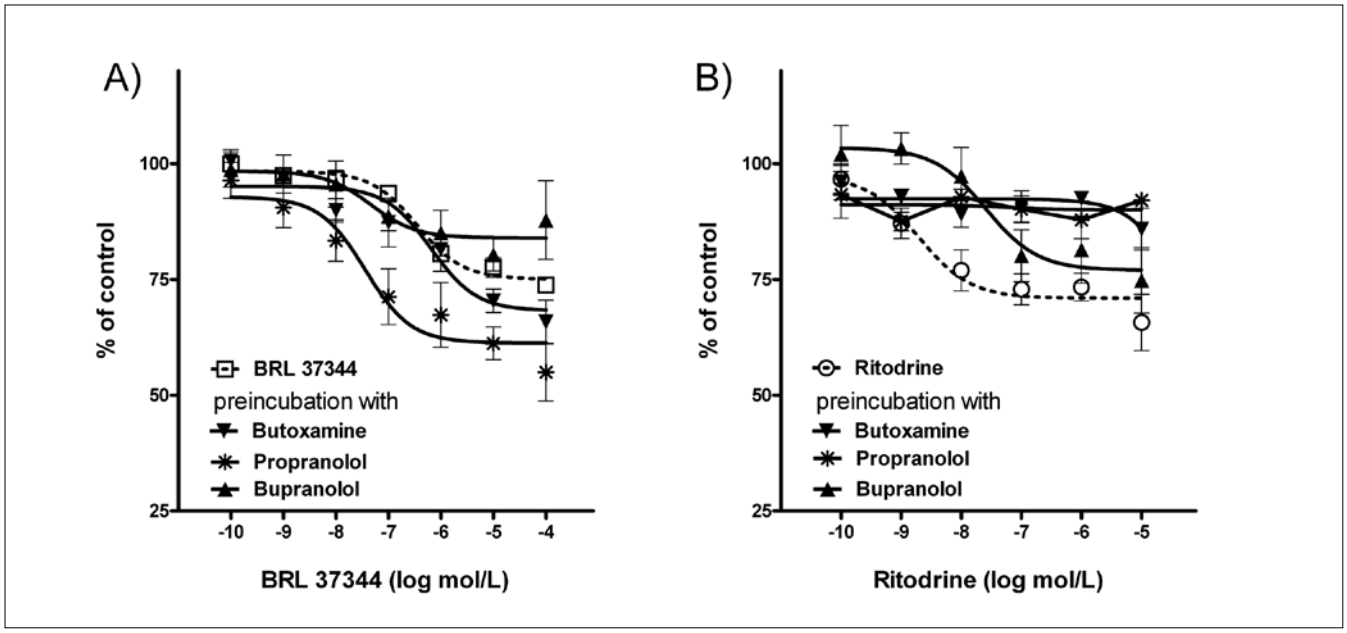

Figure 2. Concentration-response curves for cumulatively administered: A – BRL 37344 – □ (10^{-10} – 10^{-4} mol/L; N = 5), and B – ritodrine – ○ (10^{-10} – 10^{-5} mol/L; N = 13); individually and after preincubation with butoxamine – ●, propranolol – ✳, or bupranolol – ▲. Spontaneous contractions of the myometrial strips before the addition of agonist were treated as control values. Each point represents mean ± SEM.

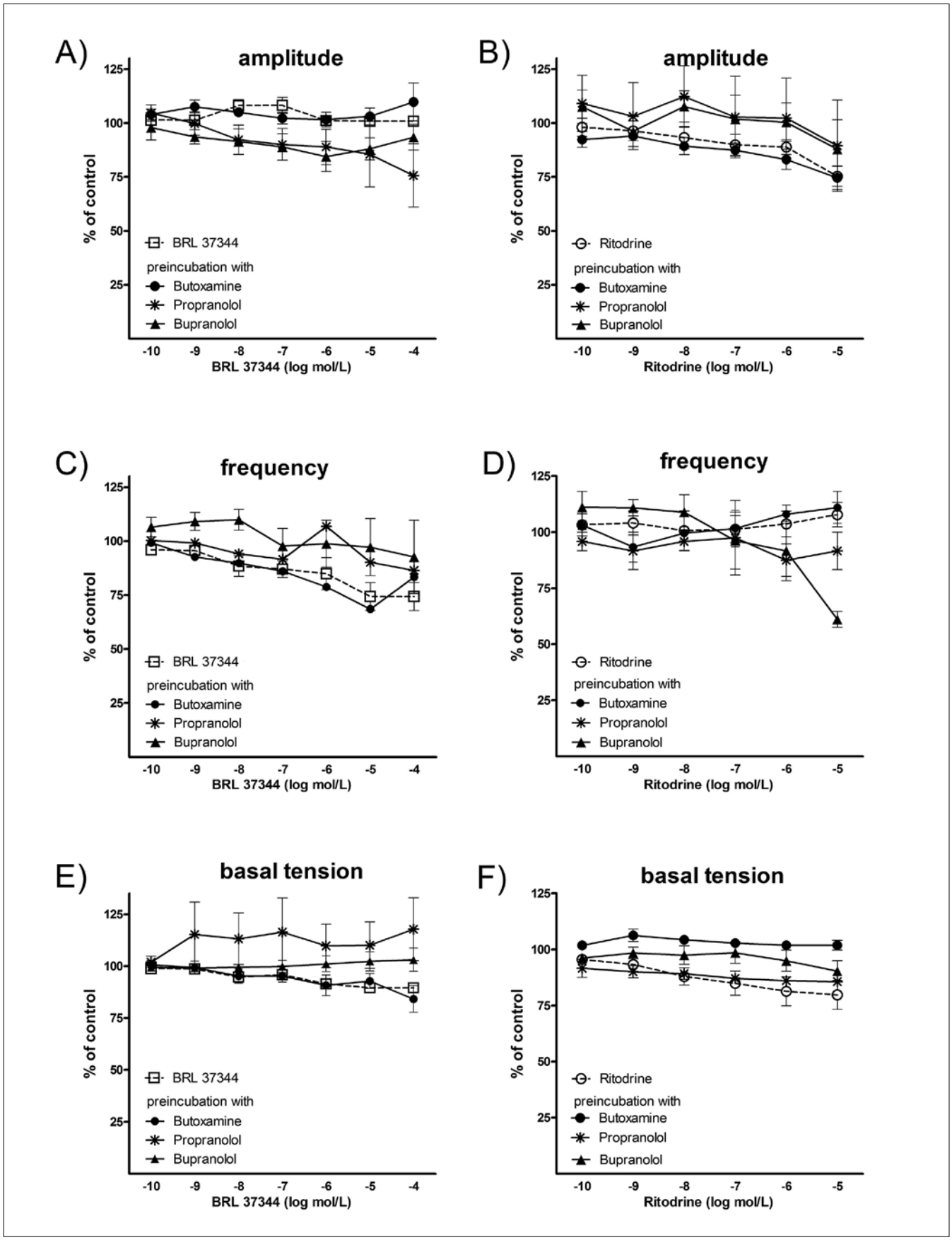

Figure 3. The cumulative effect of BRL 37344 – □ (N = 5) and ritodrine – ○ (N = 13) alone on the amplitude, frequency, and basal tension of contractions of human nonpregnant myometrial strips and the effects of β-adrenoceptors antagonists (each at 10^{-6} mol/L): butoxamine – ● (N = 7 and N = 5, respectively), propranolol – ✳ (N = 5 and N = 5, respectively), or bupranolol – ▲ (N = 6 and N = 6, respectively), on changes in these parameters The amplitude, frequency, and basal tension of spontaneous contractions before the addition of agonists were treated as control values. Each point represents mean ± SEM.

Pretreatment of the myometrial strips with propranolol abolished the concentration-dependent decrease of the mean amplitude of contractions caused by ritodrine, and also diminished the mean frequency of contractions, but did not change the effect of ritodrine on the basal tension. (Figure 3F).

Effect of bupranolol on the relaxation response to BRL 37344 and ritodrine

The presence of bupranolol in the organ bath diminished the BRL 37344-induced relaxation of myometrial strips. This effect was statistically significant in the concentration range of 10^{-7} – 10^{-4} mol/L. (Figure 2A and Table I).

Preincubation of the myometrial strips with bupranolol slightly decreased ($P < 0.05$) the mean amplitude of contractions and markedly abolished ($P < 0.05$) the influence of BRL 37344 on both the mean frequency of contractions and the basal tension. (Figures 3A, C, and E).

Preincubation of myometrial strips with bupranolol resulted in a significant rightward shift of the concentration-response curve for ritodrine. (Figure 2B and Table I).

In the concentration range of 10^{-10} – 10^{6} mol/L for ritodrine, the abolition of the ritodrine-induced decrease of the mean amplitude of contractions was observed. In the presence of bupranolol, only the highest concentration of ritodrine used was able to cause a decrease in the mean frequency of contractions. Pretreatment with bupranolol attenuated the effect of ritodrine on the basal tension. (Figure 3F).

Discussion

In spite of a possible vivid interest in the topic, only a few papers relative to the action of β_3-adrenoceptors agonists on contractile activity of the human myometrium have so far been published. The relaxation of human pregnant myometrium by BRL 37344 was first reported by Dennedy *et al.* [7]. In a subsequent paper, they concluded that BRL 37344 relaxes human pregnant myometrium via β_3-adrenoceptors activation [13]. However, precise mechanisms through which uterine contractions are induced and abolished have not been fully established [14, 15]**.** It has been hypothesized that, in the myometrium, Ca^{2+} influx through voltage-gated L-type channels is sufficient for normal spontaneous activity whilst contractile activity evoked by agonists involves Ca^{2+} release from the sarcoplasmic reticulum [16].

The present study compared the influence of β_3-adrenoceptor agonist BRL 37344 on the contractility of human nonpregnant myometrium with the effect of ritodrine – a reference compound as a tocolytic agent and a selective β_2-adrenoceptor agonist. Interestingly, BRL 37344 in a concentration-dependent manner relaxed human nonpregnant myometrium. It is also of interest to compare our data with those reported by Dennedy *et al.* [7] for the pregnant uterine muscle. Our concentration-response curves suggest that, with the maximal inhibition of ~25% (IC_{50} = -6.45 ± 0.18), BRL 37344 is less efficient in relaxing the nonpregnant human myometrium than pregnant near-term human myometrium, where the maximal inhibition of ~62% and IC_{50} = -7.26 ± 0.48 were found [7]. Such an effect is in accordance with the recently published data indicating that the expression of β_3-adrenoceptor mRNA [4], as well as the density of β_3-adrenoceptor binding sites [17] are higher in pregnant than in nonpregnant human myometrium. On the other hand, the data published by Doheny *et al.* [18] suggested that, in pregnant human myometrium, BRL 37344 attenuates to a greater degree the oxytocin-induced contractile activity than spontaneous contractile activity However, another β_3-adrenoceptor agonist, SR 59119A, has been shown to more efficiently decrease the spontaneous activity of human pregnant myometrium than that of human nonpregnant myometrium [4], data in support of our conclusion.

The position of the concentration-response curve for ritodrine to the left of the curve for BRL 37344 indicates its higher potency. Although there was no statistically significant difference in the maximal inhibition values, BRL 37344 seems to be less potent than ritodrine in relaxing nonpregnant myometrium. The analysis of amplitude, frequency and basal tension revealed the differences in the mechanisms of relaxation caused by the two β-adrenoceptor agonists. A concentration-dependent decrease of the amplitude, but not the frequency of contractions, was characteristic for the ritodrine-induced relaxation. BRL 37344, however, decreased the frequency of contractions without changing their amplitude. Both compounds diminished the basal tension of the myometrium strips.

The blockage of β_2-adrenoceptors with 10^{-6} mol/L butoxamine considerably inhibited the relaxing effect of ritodrine, mainly by preventing the decrease of basal tension. Surprisingly, butoxamine did not change the effect of ritodrine on the mean amplitude of contractions. Nevertheless, the effects exerted by the β-adrenoceptor agonists on the strips incubated with butoxamine indicate that the effect of BRL 37344 on the human nonpregnant myometrium is not mediated by β_2-adrenoceptors.

Bupranolol, the non-specific β-adrenoceptors antagonist, at concentration 10^{-6} mol/L, decreased the responses of the myometrium strips to both ritodrine and BRL 37344. At high concentrations bupranolol antagonizes both the β_2- and β_3-adrenoceptors. Thus, our results support the view that the relaxation of human nonpregnant myometrium by BRL 37344 is mediated by β_3-adrenoceptors.

Preincubation of the strips with propranolol counteracted the relaxation induced by ritodrine and caused a paradoxical leftward shift of the concentration-response curve for BRL 37344. We cannot at the moment provide any concrete explanation for the above-mentioned shift. However, it has been reported that propranolol, BRL 37344, and bupranolol demonstrate affinity to α-adrenergic receptors [19, 20]. The binding of propranolol to α-adrenergic receptors might result in an apparent increase in potency of the β_3-adrenoceptor agonist. It has also been reported that β-adrenoceptor antagonists induce changes in the electrophysiological properties of myocardial cell membranes, such as automaticity, excitability, conductivity, and refractoriness [21].

The analysis of the β-adrenoceptor agonists action on the parameters of contractile activity shows that, in the presence of propranolol, BRL 37344 causes a decrease of the amplitude of the contractions but no change in their frequency or basal tension. This finding implies the existence of a second mechanism of BRL 37344 action on the human nonpregnant myometrium, which is not related to the activation of the β_3-adrenoceptors.

From the clinical standpoint, women with primary dysmenorrhea have been shown to experience immediate pain relief following β_2-adrenoceptor agonist terbutaline administration [22, 23]. A decrease in the myometrial hyperactivity (represented

by contractions of high amplitude and long duration) has been found as the treatment's mechanism [22], and various routes of administration have been tested [23, 24]. Thus, insight into detailed means of action of particular β-adrenoceptor agonists in nonpregnant uterus could help design appropriate causative treatment for primary dysmenorrhea with minimal side effects.

Concisely, our results support the view that relaxation of human nonpregnant myometrium by BRL 37344 is mediated through β_3-adrenoceptors. A comparison of the effects of BRL 37344 and ritodrine shows that both β-adrenoceptors agonists are effective in relaxing nonpregnant myometrium, while BRL 37344 seems less potent than ritodrine. The present data imply the existence of a second mechanism of BRL 37344 action on the human nonpregnant myometrium, which is not related to the activation of the β_3-adrenoceptors.

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