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Comparison of effects of formoterol and BRL 37344 on isolated term-pregnant rat myometrial strips in vitro

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Abstract

This study was designed to compare the effects of β-adrenoceptor agonists formoterol and BRL 37344 on spontaneous contractions and the levels of cAMP and cGMP of myometrial strips isolated from timed-pregnant rats. Myometrial strips were obtained from term-pregnant Wistar albino rats ($n=12$), mounted in organ baths and tested for changes in isometric tension in response to formoterol and BRL 37344. We evaluated the effect of increasing concentrations of formoterol and BRL 37344 on oxytocin-induced myometrial contractions and on contractions of myometrial smooth muscle pretreated with metoprolol, ICI 118.551 and SR 59230A (β_1, β_2, β_3-adrenoceptor antagonist, respectively, 10^{-6} M). Effects of formoterol and BRL 37344 on cAMP and cGMP levels in isolated myometrial strips ($n=6$) were evaluated by radioimmunoassay kits. Formoterol (10^{-12}–10^{-8} M) and BRL 37344 (10^{-11}–10^{-5} M) concentration-dependently decreased the amplitude of oxytocin-induced contractions. E_{max} value (100%) of formoterol was increased significantly more than E_{max} value (70.6%) of BRL 37344 ($P<0.05$), with no change in pD_2 value (9.54±0.12 and 9.12±0.12, respectively). The inhibition of the amplitude of oxytocin-induced contractions by formoterol was antagonized with ICI 118.551 (10^{-6} M), but they were not changed by metoprolol (10^{-6} M) or SR 59230A (10^{-6} M). The inhibition of the amplitude of oxytocin-induced contractions by BRL 37344 were antagonized with SR 59230A (10^{-6} M), but they were not changed by metoprolol (10^{-6} M) or ICI 118.551 (10^{-6} M). Formoterol and BRL 37344 increased cAMP levels. BRL 37344 increased cGMP levels in BRL 37344 group more than control group, but this increase is less significant than cAMP levels ($P>0.05$). Formoterol and BRL 37344 decreased amplitude of myometrial contractions with similar potency, but efficacy of formoterol was better than BRL 37344.

Keywords: Formoterol; BRL 37344; Preterm labor; cAMP; cGMP; (Rat)

1. Introduction

Preterm labor, initiation of birth prior to the 37th week of gestation, is a major public health problem worldwide and many cases cannot be explained on the basis of associated risk factors, let alone physiological mechanisms (Buxton et al., 2000; Pacora et al., 2002). Preterm labor complicates 8–10% of all pregnancies (Berkowitz and Papiernik, 1993). Spontaneous preterm labor accounts for 40–50% of all preterm deliveries, with the remainder resulting from preterm premature rupture of membranes (25–40%) and obstetrically indicated preterm delivery (20–25%) (Tucker et al., 1991).

Prevention of preterm birth continues to be an important subject for the obstetrician. Despite many advancements in perinatal care and approach obstetric treatment, spontaneous preterm delivery remains a leading cause of neonatal morbidity and mortality, and preterm infants commonly encounter life-long complications.

Cornerstone in treatment of preterm labor remains a pharmacological inhibition of uterine contractions by tocolytic drugs. Tocolytic therapy may offer some short-term benefit in the management of preterm labor. The goals of tocolitic therapy are to prolong pregnancy and enhance fetal lung development

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by administering corticosteroids and reduce the severity of fetal respiratory distress syndrome. Inhibitors of smooth muscle contraction, like β_2-adrenoceptor agonists, magnesium sulphate, and calcium channel blockers, are the most important agents used for the treatment of preterm delivery but their efficacy and safety are controversial (Keirse, 1995; Kantas et al., 2002; Yarar et al., 2001). The delay can also be used to facilitate transfer of the patient to a tertiary care center. New drugs which allow more effective treatment of preterm labor with lower side effects are needed.

Formoterol hemifumarate is a highly potent, long-lasting β_2-adrenoceptor agonist that is used for asthma therapy. Specific β_2-adrenoceptor agonist is a popular group of drugs employed for countering preterm labor, although in their conventional form, such as ritodrine, they are relatively short acting (Keirse, 1995).

The more recently described β_3-adrenoceptor (Strosberg and Pietri-Rouxel, 1996) has many functions in different human tissues but it is intimately linked to smooth muscle relaxation in gastrointestinal (Hutchinson et al., 2000), urinary tract (Longhurst and Levendusky, 1999), respiratory (Mustafa et al., 1999) and vascular smooth muscle systems (Trochu et al., 1999). The novel β_3-adrenoceptor agonists are thought to activate primarily the β_3-adrenoceptor (Oriowo et al., 1996).

In the present study, we attempt to compare the actions of β_2-adrenoceptor agonist formoterol ((±)-(*R**,*R**)-*N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl] amino]ethyl]phenyl] formamide hemifumarate) and β_3-adrenoceptor agonist BRL 37344 ((*R**,*R**)-(±)-4-[2-[(2-(3-Chlorophenyl)-2-hydroxyethyl)amino]propyl] phenoxyacetic acid, sodium salt) on oxytocin-induced contractions and levels of cAMP and cGMP of myometrial strips isolated from timed-pregnant rats.

2. Materials and methods

2.1. Animals and myometrial tissues preparation

Wistar albino rats ($n=18$) at the 19th or 21st day of gestation (term-pregnant rat), weighing 180–220 g, were used throughout the study. Ethics committee approval for the study was obtained from the Animals Research Ethics Committee at Cumhuriyet University School of Medicine. Rats were housed in a 22 °C temperature room with water and food ad libitum. Virgin female rats were placed in separate cages with one male each and left overnight. Copulatory plugs were examined and obtained vaginal smear specimen on the next morning after pairing. A pediatric otoscope (HEINE mini 2000, Heine Optotechnik, Herrsching, Germany) was used to demonstrate the copulatory plug. We obtained vaginal smear specimen that contain spermatozoa and was designated as day 0 of gestation (Voipio and Nevalainen, 1998).

Pregnant rats were killed by cervical subluxation at gestational day 21. A midline abdominal incision was made; the uterine horns were immediately excised, and carefully cleaned of surrounding connective tissues, then opened longitudinally along the mesenteric border. Pups were removed and non-uterine tissues were dissected away. We incubated myometrial tissues in 10 ml organ baths at 37 °C (pH=7.4) containing modified Krebs–Henseleit solution (NaCl 125 mM, KCl 2.4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.5 mM, $NaHCO_3$ 23.9 mM and glucose 11.0 mM) aerated continuously with 95% O_2 and 5% CO_2.

2.2. Myometrial tissue bath experiments

The myometrial tissues obtained from each term-pregnant rats ($n=12$) and dissected into six full-thickness longitudinal muscle strips (approximately 8×2×2 mm) and then myometrial tension was recorded isometrically with a Grass FT03 force-displacement transducer and registered on a Grass model 79E polygraph (Grass, Quincy, MA, USA). The recorder was calibrated as 10 mN tension was represented as 1 cm vertical displacement. The strips in tissue baths were allowed to equilibrate for at least 1 h. The Krebs–Henseleit physiologic salt solution in the tissue baths was changed every 15 min during the equilibration period. After equilibration, contractions were stimulated by adding oxytocin (10 mU/ml) into the baths containing strips to for a period of 30 min, until regular phasic contractions were achieved. The β_2-adrenoceptor agonist formoterol (10^{-12}–10^{-8} M) or the β_3-adrenoceptor agonist BRL 37344 (10^{-11}–10^{-5} M) was then added to the bath in a cumulative manner. The cumulative increases in bath concentration of formoterol or BRL 37344 were achieved in a pattern of one log molar increase every 15 min. Then, bath addition of the following β-adrenoceptor antagonists was performed for a 30-min period: metoprolol (β_1-adrenoceptor antagonist; 10^{-6} M), ICI 118.551 ((±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl) oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride, β_2-adrenoceptor antagonist; 10^{-6} M), SR 59230A (1-(2-ethylphenoxy)-3-[[(1*S*)-1,2,3,4-tetrahydro-1-naphthalenyl]amino]-(2*S*)-2-propanol hydrochloride, β_3-adrenoceptor antagonist; 10^{-6} M) or their respective vehicle for each antagonist. It was followed by the addition of the β_2-adrenoceptor agonist formoterol or β_3-adrenoceptor agonist BRL 37344, in a cumulative manner, at bath concentrations in 15-min intervals.

2.3. Measurement of cAMP and cGMP levels in myometrial strips

Myometrial tissues (6×2×2 mm) were obtained from term-pregnant rats ($n=8$) and dissected in to 10 full-thickness longitudinal muscle strips (approximately 6×2×2 mm). The myometrial strips were equilibrated in Krebs solution (composition as above) continuously aerated with a mixture of 95% oxygen and 5% carbon dioxide at 37 °C (pH 7.4) for 60 min. Four sets of experimental studies were performed except for control experiments. In four sets for formoterol, four myometrial strips were isolated from each rat. Myometrial strips were stimulated with oxytocin (10 mU/ml) for 15 min in all sets. Tissues were exposed to formoterol hemifumarate for 15 min in the first set. In the second, third, and fourth sets, tissues were exposed to antagonist metoprolol (10^{-6} M), ICI 118.551 (10^{-6} M) and SR 59230A (10^{-6} M), respectively, before incubation with