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Comparative relaxant effects of YC-1 and DETA/NO on spontaneous contractions and the levels of cGMP of isolated pregnant rat myometrium

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Abstract

This study was designed to compare the effects of YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole), a nitric oxide (NO)-independent soluble guanylate cyclase activator, and diethylenetriamine-NONOate (DETA/NO), a NO donor, on spontaneous contractions and the levels of cyclic GMP (cGMP) of myometrial strips isolated from timed-pregnant rats. Myometrial strips were obtained from timed-pregnant Wistar albino rats ($n=10$) and were mounted in organ baths and tested for changes in isometric tension in response to YC-1 and DETA/NO. We also evaluated the effect of YC-1 and DETA/NO on the levels of cGMP in myometrial strips obtained from timed-pregnant rat uterine horns ($n=20$). YC-1 (10^{-9}–3×10^{-5} M) and DETA/NO (10^{-7}–10^{-4} M) concentration-dependently decreased the amplitude and frequency of spontaneous contractions of myometrial strips isolated from term-pregnant rats. The inhibitions of the amplitude and frequency of spontaneous contractions by YC-1 and DETA/NO were antagonized with methylene-blue (10^{-5} M). Antagonistic effect of methylene-blue (10^{-5} M) was more on DETA/NO responses than that of YC-1 ($P<0.05$). In addition, YC-1-stimulated myometrial strips showed more elevation in myometrial cGMP than that of DETA/NO ($P<0.05$). We demonstrated that YC-1 and DETA/NO induce relaxations in the amplitude and frequency of spontaneous contractions of myometrial strips with different potencies. We also found that YC-1 and DETA/NO-induced relaxations are associated with significant increases in cGMP. These results might suggest that the relaxant effects of YC-1 and DETA/NO on the rat myometrium could be due to the stimulation of the soluble guanylate cyclase and cGMP may play a role for the maintenance of uterine quiescence during pregnancy.

Keywords: YC-1; DETA/NO; Soluble guanylate cyclase activator; Nitric oxide donor; Myometrium

1. Introduction

Prematurity is one of the major unresolved problems in obstetrics. Neonatal mortality and morbidity associated with premature labor have stimulated a search for new agents those diminish or eliminate uterine contraction. Although tocolytics based on β-adrenergic receptor and calcium channel antagonists are in use, their efficacy and safety are questionable (Koks et al., 1998). Knowledge of the actions of nitric oxide (NO) as a vascular and gastrointestinal smooth muscle relaxant together with initial clinical evidence has suggested the use of NO in the treatment of preterm labor.

NO is an important mediator of many biologic functions, including the relaxation of smooth muscle of vascular, gastrointestinal, tracheal, and cavernous tissues (Moncada et al., 1991; Li and Rand, 1990; Ozaki et al., 1992; Li and Rand, 1991; Pickard et al., 1991). It is an activator of soluble guanylate cyclase and is effective agent in relaxing spontaneous contractions of both animal and human myometrium (Kuenzli et al., 1996; Kuenzli et al., 1998; Bradley et al., 1998). Several studies provide evidence for the presence of an L-arginine-NO system in the pregnant uterus (Yallampalli et al., 1993; Yallampalli et al., 1994;

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Buhimschi et al., 1995; Sladek and Roberts, 1996). Furthermore, this system is up regulated during pregnancy, and it inhibits uterine contractility until term but not during delivery, suggesting that the NO system may contribute to the maintenance of uterine quiescence during pregnancy and the initiation of labor (Yallampalli et al., 1994). It has been claimed that NO promotes human uterine relaxation, as it does other smooth muscle, by the elevation of cyclic guanosine monophosphate (cGMP) (Yallampalli et al., 1993; Buhimschi et al., 1995; Izumi and Garfield, 1995). However, several other studies have demonstrated that a rise in cGMP fails to cause a relaxation of some smooth muscles, including human myometrium (Kuenzli et al., 1996; Bradley et al., 1998; Buxton et al., 2001).

Besides NO, only few other soluble guanylate cyclase-activating substances have been reported. In 1995, the benzylindazole derivative YC-1 ([3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole]) was described as a novel, apparently NO-independent activator of soluble guanylate cyclase (Wu et al., 1995). It is a direct activator of soluble guanylate cyclase and sensitizes the enzyme for activation by NO and carbon monoxide (Schmidt et al., 2001). YC-1 provides an about 10-fold increase of enzyme activity (Koesling and Friebe, 1999). Apart from an increase in the formation of cGMP via the stimulation of soluble guanylate cyclase, the substance also prevents cGMP degradation. In our previous study, we demonstrated that YC-1 inhibits spontaneous contractions of myometrial strips obtained from timed-pregnant rat uterus and methylene-blue (which blocks soluble guanylate cyclase) or tetraethylammonium (which blocks Ca^{2+}-activated K^+ channels) antagonizes the inhibitor effect of YC-1 (Cetin et al., 2004).

In the present study, we attempt to compare the actions of YC-1 and DETA/NO on spontaneous contractions and levels of cGMP of myometrial strips isolated from timed-pregnant rats.

2. Material and methods

2.1. Animals

Timed-pregnant ($n=10$) Wistar albino rats obtained from Cumhuriyet University Animal House, weighing 180–200 g, were used throughout the study. After obtaining Institutional Review Board approval, all procedures were performed under the guidelines of the Animal Care and Use Committee of Cumhuriyet University. 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. Pregnancy was dated by accepting the morning of sperm positivity as day one of gestation. The normal length of gestation of rats was 22 days. None of the rats used was in labor. Pregnant rats were killed by cervical subluxation at 21 days of gestation. A midline abdominal incision was made; the uterine horns were rapidly excised, carefully cleaned of surrounding connective tissues, and then opened longitudinally along the mesenteric border. Fetuses were removed and non-uterine tissues were dissected away. We obtained five full-thickness longitudinal muscle strips (approximately $8\times2\times2$ mm) from each animal and incubated them in temperature-controlled 10 ml organ baths containing modified Krebs' 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 with 95% O_2 and 5% CO_2 at 37 °C (pH=7.4).

2.2. Measurement of myometrial contractile activity

The myometrial strips were allowed to equilibrate at 1 g tension for 60 min before the addition of the experimental drugs and washed every 15 min. The 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 so that 1 g tension was represented as 1 cm vertical displacement. Paper speed was set at 2.5 mm/min. Myometrial contractions started within 10 min after they were mounted in the organ bath and stabilized in 60 min. Preliminary time-control experiments with no further drug additions showed that strips exhibit stable uterine activity for at least 4 h after preparation in this manner.

Three sets of experimental studies were performed with myometrial strips obtained from pregnant rats ($n=10$). While conducting the three sets of studies, we used the three myometrial strips isolated from each rat. The effect of YC-1 (10^{-9}–3×10^{-5} M) on the spontaneous contractions alone and in the presence of methylene blue (10^{-5} M) was evaluated in the first. In the second set, we evaluated the effect of DETA/NO (10^{-7}–10^{-4} M) alone and in the presence of methylene blue (10^{-5} M) on the spontaneous contractions of rat myometrium. Methylene blue was added to the organ baths 15 min before from YC-1 and DETA/NO in order to test the role of guanylate cyclase which could have a contribution to myometrial smooth muscle relaxation induced by YC-1 and DETA/NO. In the third set, we determined the effect of dimethyl sulfoxide (DMSO) that have used as a solvent of the YC-1 on the spontaneous contractions of rat myometrium.

2.3. Determination of myometrial cGMP content

To determine the myometrial cGMP content of rat myometrial strips under experimental conditions, 80 myometrial strips isolated from term-pregnant rats were used ($n=20$). They were equilibrated for 60 min in Krebs–Henseleit bicarbonate buffer at 37 °C, and then incubated for a further 1, 5, 20, or 60 min with YC-1 (10^{-6} M, $n=4$, and 10^{-5} M, $n=4$), DETA/NO (10^{-6} M, $n=4$, and 10^{-5} M, $n=4$), and DMSO ($n=4$). Strips were collected either immediately by freezing the strips in liquid nitrogen. All strips were pulverized in a liquid nitrogen-cooled stainless steel mortar, and then transferred into 300 μl of 80% ethanol. From this point on, cyclic GMP standards were processed and treated identically to the samples. Samples were homogenized with ethanol and incubated for 30 min at 4 °C. Then, they were centrifuged at 4 °C for 10 min at 12,000 $\times g$ to precipitate cell debris and proteins. To remove ethanol, we poured the supernatants into a clean test tube and dried the supernatant in a vacuum at 50 °C. The 100 μl aliquot of these supernatant fractions was used for cGMP quantitation by radioimmunoassay (IBL, Hamburg, Germany). The amount of cGMP in each myometrial strip was standardized to fmol cGMP mg^{-1} protein.