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Short communication

Effects of nimodipine and isradipine on endothelin-1-induced contraction of pregnant rat myometrium

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Abstract

The purpose of this study was to investigate the effect of nimodipine and isradipine on endothelin-1-induced contractions of isolated pregnant rat myometrium. Endothelin-1 at 10^{-10}–10^{-8} M dose-dependently increased the amplitude and frequency of contractions and decreased the duration of contractions. Basal tone of myometrium was increased with the higher endothelin-1 concentrations (10^{-9} and 10^{-8} M). After pretreatment for 30 min with 10^{-6} M nimodipine or 10^{-5} M isradipine, amplitude and duration of endothelin-1-induced contractions were significantly decreased, but frequency of myometrial contractions increased markedly. However, neither agent abolished the stimulating effect of higher endothelin-1 concentrations (10^{-9} and 10^{-8} M) on the basal tone. Our study showed that phasic activity is inhibited by nimodipine and isradipine, whereas tonic activity is not inhibited. The contraction of myometrial strips isolated from the pregnant rat may be modulated by endothelin-1, and this effect is only partly modulated by dihydropyridine-type Ca^{2+} channels. The remaining resistance to nimodipine- and isradipine-induced inhibition may be explained by pregnancy-associated changes in the other electrophysiological and biochemical factors. © 1998 Elsevier Science B.V.

Keywords: Endothelin-1; Nimodipine; Isradipine; Myometrium; Rat; Pregnant

1. Introduction

Endothelin-1 is a vasoconstrictor peptide of endothelial origin belonging to a family of four isopeptides consisting of endothelin-1, endothelin-2, endothelin-3 and vasoactive intestinal constrictor, or endothelin-4 (Yanagisawa et al., 1988; Aardal, 1994). Subsequent studies showed that these peptides are also found in non-epithelial cells from a variety of tissues such as adrenal gland, breast and myometrium. It has been suggested that endothelins may cause vascular smooth muscle contraction via activation of voltage-dependent Ca^{2+} channels (Yanagisawa et al., 1988). Further, it has been demonstrated that endothelin stimulates contraction of myometrium by a partly dihydropyridine-sensitive mechanism (Fried et al., 1993). It has been demonstrated that endothelin-1 and endothelin-2 have equal potency to affect contractile responses, whereas endothelin-3 was considerably less potent than endothelin-1 or endothelin-2. Endothelins induce two types of myometrial contraction, one, spontaneous contractile activity and one, basal tone (Kozuka et al., 1989).

Myometrial smooth muscle appears to be activated primarily by the influx of Ca^{2+} from the extracellular fluid. This transfer of extracellular Ca^{2+} to the intracellular space is via voltage- or receptor-operated channels. The inhibition of myometrial contractions with Ca^{2+} channel blockers, a group of heterogeneous agents, is mediated primarily by the voltage-dependent channels. In vitro studies using myometrial strips have been generally limited to nitrendipine and nifedipine (Odum and Pipkin, 1988; Saade et al., 1994). In addition, there have been no studies on the effect of nimodipine and isradipine on the contractile response of rat myometrium. The endothelins appear to play a fundamental role in the control of uterine function in pregnancy and in the mechanisms governing the onset of preterm labor. Since Ca^{2+} channel blockers show great promise in treating preterm labor and since experimental data about nimodipine and isradipine are too sparse to

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allow use in preterm labor at this time, we examined the effect of nimodipine and isradipine on endothelin-1-induced contractions of myometrium isolated from the pregnant rats.

2. Materials and methods

Day 18, timed-pregnant Albino rats (200 to 250 g) ($n = 12$) were cared for according to the guidelines of the Cumhuriyet University, at the Animal Care Center. The animals were killed by cervical subluxation. The uterine horns were rapidly excised and carefully cleaned of surrounding connective tissue and opened longitudinally along the mesenteric border. Fetuses were removed from the late-stage pregnant rats and non-uterine tissues were dissected away and discarded. The myometrium was cut into longitudinal strips approximately 10 mm × 3 mm × 3 mm, following muscle orientation, and were mounted vertically in a 10-ml organ bath containing modified Krebs solution (composition in millimoles per liter: sodium chloride 125, potassium chloride 2.4, calcium chloride 1.8, magnesium chloride 0.5, sodium bicarbonate 23.9, and glucose 11) aerated with 95% oxygen and 5% carbon dioxide at 37°C.

Myometrial tension was recorded isometrically with a Grass FT03 force-displacement transducer and recorded on a Grass model 79 E polygraph (Grass, Quincy, MA, USA). The myometrial strips were suspended at 1 g tension for 30 min before the addition of the experimental drugs. Recorder paper speed was set at 5 mm/min and calibrated so that 1 cm of vertical displacement represented 1 g of tension.

Concentration–response curves for endothelin-1 (10^{-11}–10^{-8} M), nimodipine (10^{-8}–10^{-4} M), and isradipine (10^{-8}–10^{-4} M) were obtained on spontaneous myometrial contractions at the beginning of the study. Endothelin-1 (10^{-10}–10^{-8} M) dose-dependently increased the contractile activity of myometrial strips, reaching statistical significance at a endothelin-1 concentration of 10^{-9} M ($P < 0.05$, $n = 6$). Nimodipine and isradipine, starting at concentrations of 10^{-6} and 10^{-5} M, respectively, significantly decreased spontaneous myometrial contractions

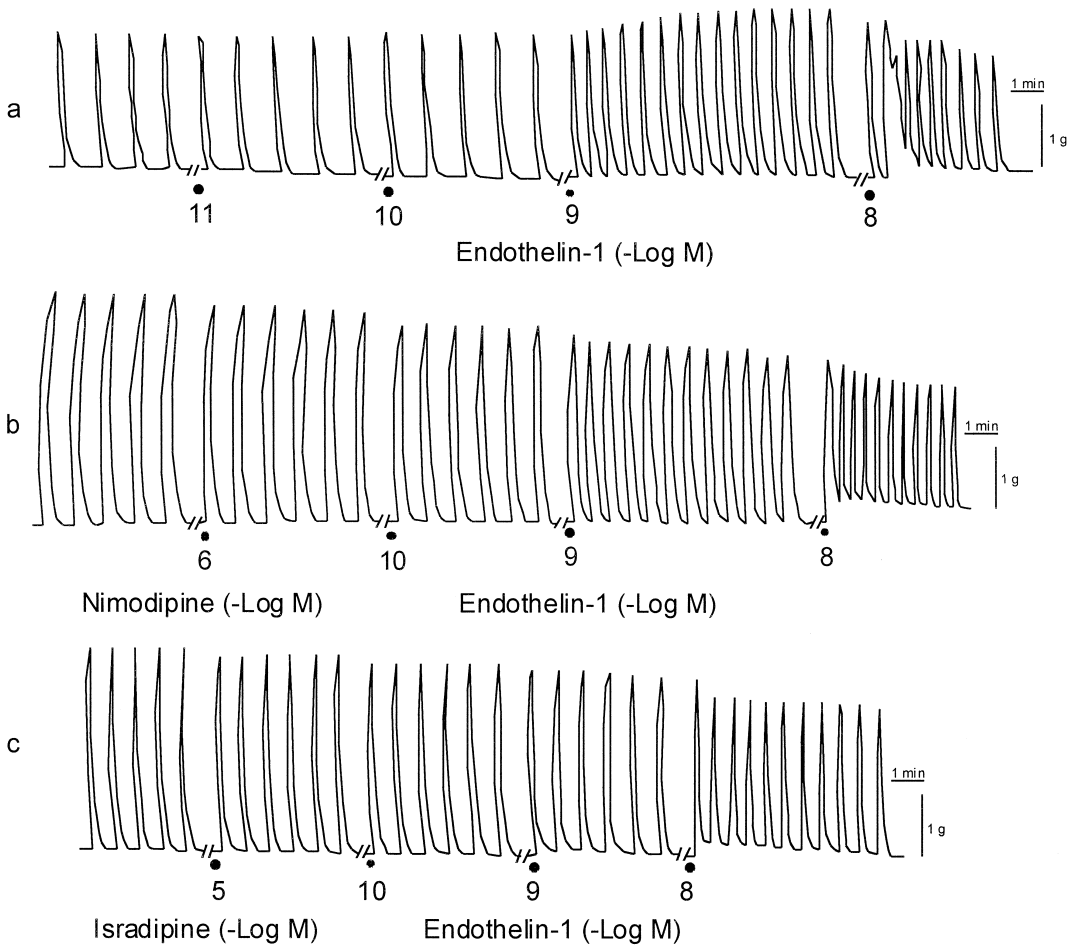

Fig. 1. (a) Effect of endothelin-1 (10^{-11}–10^{-8} M) on spontaneous contractile activity of myometrium isolated from pregnant rats ($n = 12$). (b) Effect of endothelin-1 on myometrial strips ($n = 6$) pretreated for 30 min with nimodipine (10^{-6} M). Increasing concentrations of endothelin-1 ranged from 10^{-10} to 10^{-8} M were added at 1000-s intervals. (c) Effect of endothelin-1 on myometrial strips ($n = 6$) pretreated for 30 min with isradipine (10^{-5} M). Increasing concentrations of endothelin-1 ranged from 10^{-10} to 10^{-8} M were added at 1000-s intervals.

($P < 0.05$, $n = 6$). Both nimodipine and isradipine at a concentration of 10^{-4} M inhibited spontaneous contractions completely. Two sets of experiments were performed. In the first set, cumulative dose–response curves were generated by exposing myometrial strips ($n = 6$) pretreated for 30 min with nimodipine, 10^{-6} M, to increasing doses of endothelin-1 ranging from 10^{-10} to 10^{-8} M. In the second set, cumulative dose–response curves were generated by exposing the myometrial strips ($n = 6$) pretreated for 30 min with isradipine, 10^{-5} M, to increasing doses of endothelin-1 ranging from 10^{-10} to 10^{-8} M.

Chemicals used in the current experiments were endothelin-1 purchased from Sigma (St. Louis, MO, USA) and nimodipine and isradipine obtained from ICN (Costa Mesa, CA, USA). Drug-containing solutions were made in 0.9% NaCl and added to the bath in volumes of 50 μl.

The characteristics of the contractions analyzed immediately before and after the addition of drugs included mean amplitude (in grams), frequency (number per 1000 s), and duration (in seconds) of each contraction for 1000-s intervals. The change in basal tone after addition of the endothelin-1 was estimated as the change in maximum contraction as a percentage of the maximal contraction elicited before the addition of endothelin-1 for each strips. Data are presented as means ± S.E.M. and were analyzed with the Mann–Whitney test and analysis of variance with Newman–Keuls test when appropriate, and $P < 0.05$ was considered significant.

3. Results

Endothelin-1 (10^{-10}–10^{-8} M) dose-dependently increased the contractile activity of myometrial strips ($n = 12$). The amplitude of endothelin-1-induced contractions increased over the control in a dose-dependent manner, reaching statistical significance at 10^{-9} M ($P < 0.05$). The increase in amplitude after endothelin-1 at 10^{-9} M was 23.1 ± 2.4% over the control ($P < 0.05$), whereas endothelin-1 at 10^{-8} M produced a 12.6 ± 4.6% decrease in amplitude over the control ($P < 0.05$). Endothelin-1 at 10^{-9} and 10^{-8} M produced a significant increase (59.4 ± 13.7 and 110.0 ± 13.5%, respectively) in the frequency of contractions ($P < 0.05$). The duration of contractions decreased significantly over the control (39.3 ± 3.7% and 54.1 ± 5.9%) at endothelin-1 concentrations of 10^{-9} and 10^{-8} M, respectively ($P < 0.05$) (Fig. 1AFig. 2). The basal tone of myometrial strips increased over the control at endothelin-1 concentrations of 10^{-9} and 10^{-8} M ($P < 0.05$). The increase in basal tone at 10^{-9} M endothelin-1 was 5.2 ± 1.1% of the maximal amplitude preceding the addition, whereas the endothelin-1 concentration of 10^{-8} M produced a 12.2 ± 2.2% increase in basal tone (Fig. 1A). The contractile responses were significantly reduced in strips washed with modified Krebs solution ($P < 0.05$).

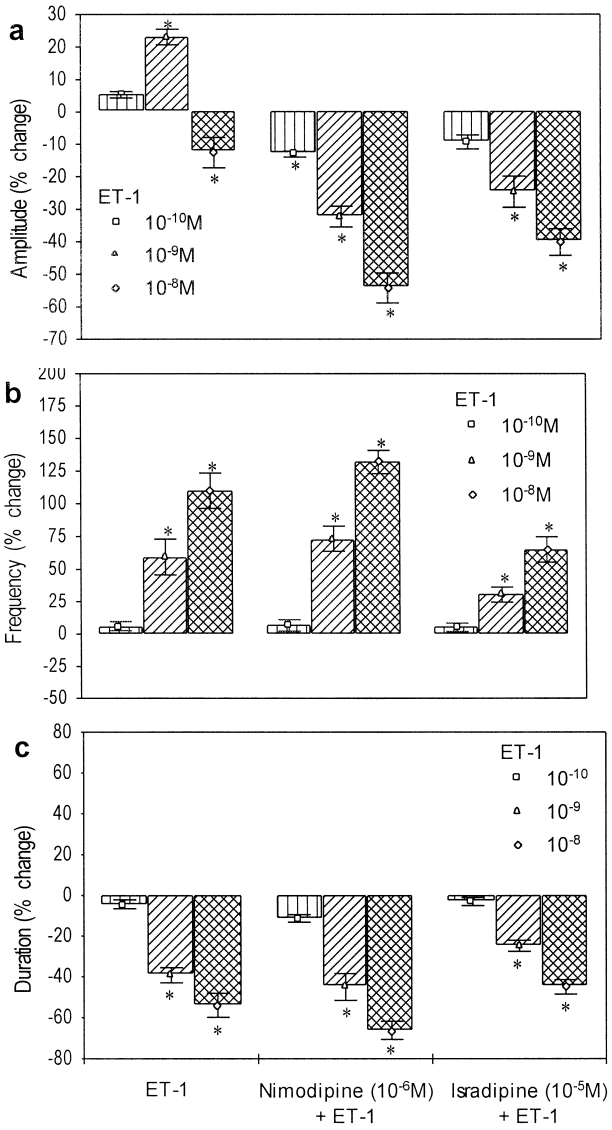

Fig. 2. Effects of endothelin-1 alone, endothelin-1 in presence of nimodipine (10^{-6} M), and endothelin-1 in presence of isradipine (10^{-5} M) on amplitude (a), frequency (b) and duration (c) of contractions of myometrium isolated from pregnant rats ($n = 6$). Data (means ± S.E.M.) expressed relative to control for effect of endothelin-1 alone, nimodipine (10^{-6} M) for effect of endothelin-1 in the presence of nimodipine (10^{-6} M), and isradipine (10^{-5} M) for endothelin-1 in the presence of isradipine (10^{-5} M) (asterisk, $P < 0.05$).

3.1. Effect of endothelin-1 on contractions of isolated pregnant rat myometrium pretreated with nimodipine (10^{-6} M)

Nimodipine starting at a concentration of 10^{-6} M decreased the spontaneous myometrial contractions during the concentration–response curve. With myometrial strips ($n = 6$) pretreated for 30 min with nimodipine (10^{-6} M), the increase in contraction amplitude induced by endothelin-1 given alone as increasing doses ranging from 10^{-10} to 10^{-8} M at 1000-s intervals was abolished. The ampli-

tude of contractions also decreased (13.1 ± 0.9%, 32.3 ± 3.2%, 54.2 ± 4.6%, respectively) significantly ($P < 0.05$) after addition of endothelin-1 at 10^{-10} to 10^{-8} M. After addition of endothelin-1 at 10^{-9} and 10^{-8} M, the frequency of contractions was significantly increased (73.3 ± 9.5% and 131.9 ± 8.8%, respectively) and the duration of contractions was significantly decreased (45.1 ± 6.6% and 66.3 ± 4.4%, respectively) ($P < 0.05$) (Fig. 1B and Fig. 2). However, the increase in basal tone seen at higher endothelin-1 concentrations (10^{-9} and 10^{-8} M) was not abolished in the presence of 10^{-6} M nimodipine (Fig. 1B).

3.2. Effect of endothelin-1 on contractions of isolated pregnant rat myometrium pretreated with isradipine (10^{-5} M)

Isradipine starting at a concentration of 10^{-5} M decreased the spontaneous myometrial contractions during the concentration–response curve. The amplitude and duration of contractions was significantly reduced, whereas the frequency was increased ($P < 0.05$). In the myometrial strips (n = 6) pretreated for 30 min with isradipine (10^{-5} M) with the addition of increasing doses of endothelin-1 ranging from 10^{-10} to 10^{-8} M at 1000-s intervals, the increase in contraction amplitude induced by endothelin-1 given alone was abolished. After addition of endothelin-1 at 10^{-10} to 10^{-8} M, the amplitude of contractions was also decreased (24.6 ± 4.8% and 40.1 ± 4.1%, respectively) significantly ($P < 0.05$). After addition of endothelin-1 at 10^{-9} and 10^{-8} M, the frequency of contractions was significantly increased (30.3 ± 5.9% and 65.1 ± 9.6%, respectively) and the duration of contractions was significantly decreased (24.8 ± 2.8% and 45.1 ± 3.6%, respectively) ($P < 0.05$) (Fig. 1C and Fig. 2). However, the increase in basal tone seen at higher endothelin-1 concentrations of 10^{-9} and 10^{-8} M was not abolished in the presence of isradipine (10^{-5} M) (Fig. 1C).

4. Discussion

Endothelin-1 is a potent uterotonic agent capable of inducing contractions in both pregnant and nonpregnant myometrium obtained from human or from rat uterus (Sakata and Karaki, 1992; Word et al., 1992). Word et al. (1990) reported that endothelin promotes contraction in the myometrium by causing an increase in intracellular Ca^{2+} through stimulation of Ca^{2+} influx as well as Ca^{2+} release from intracellular stores, and an increase in myosin light chain phosphorylation. The basal contractions of the myometrial strips, as well as the contractile effect of endothelin-1, were dependent on the presence of external Ca^{2+} (Sakata et al., 1989, Word et al., 1990). In the absence of Ca^{2+} in the buffer medium of myometrial cells, the effects of endothelin on intracellular Ca^{2+} and myosin light chain phosphorylation are decreased but not abolished (Word et al., 1990).

The present study showed that endothelin-1 affects the phasic and tonic contractile activity of isolated myometrial strips of pregnant rat. In a dose-dependent manner, endothelin-1 increases the amplitude and frequency of spontaneous contractions and decreases the duration of each contraction. In addition, the basal tone of the myometrium was increased at higher concentrations of endothelin-1.

We examined the effect of nimodipine and isradipine, 1–4 dihydropyridine-type Ca^{2+} channel blocking agents, on endothelin-1-induced contractions in myometrium isolated from the pregnant rat. After the pretreatment with either nimodipine or isradipine, the endothelin-1-induced phasic contractile activity decreased in the myometrial strips. Amplitude and duration of contractions were significantly decreased, but their frequency increased markedly. However, neither agent inhibited the stimulating effect of higher concentrations of endothelin-1 on the basal tone. In the presence of nimodipine and isradipine, endothelin-1 inhibited phasic contractions, but did not totally abolish contractile activity.

The main site of action of the Ca^{2+} channel blockers is believed to be the voltage-dependent channels, where inhibition of the influx of extracellular Ca^{2+} results in uncoupling of excitation and contraction. Calcium channel blockers may not only directly decrease the concentration of cytoplasmic Ca^{2+}, but also cause a decrease in Ca^{2+} release from intracellular stores.

The Ca^{2+} channel types described for smooth muscle consist of the L- and T-types, with slow and fast inactivation characteristics respectively (Hess, 1990). L-type Ca^{2+} channels are inhibited by Mg^{2+} and by dihydropyridines such as verapamil, nifedipine, nimodipine and isradipine (Speralakis et al., 1992; Mironneau, 1993). The function of L-type Ca^{2+} channels can be regulated both by guanosine 5′-triphosphate (GTP)-binding proteins and by phosphorylation, the latter often having a negative effect on smooth muscle (Birnbaumer et al., 1990; Sanborn et al., 1993).

In the present study, the increase induced by endothelin-1 in the contractile activity of pregnant rat myometrium was partly decreased by nimodipine and isradipine, whereas the increase in basal tone induced with endothelin-1 remained unchanged. The results indicate that the contractility of pregnant rat myometrium may be modulated by endothelin-1, and that the effects of endothelin-1 on pregnant rat myometrium are only partly mediated by dihydropyridine-sensitive Ca^{2+} channels.

The concentration-dependent decrease in amplitude and increase in frequency seen after exposure to endothelin-1 in the presence of nimodipine and isradipine may be explained as an effect of the inhibition of phasic activity in combination with an absence of inhibition of tonic activity at all the concentrations as well as an increase in tonic activity at the higher concentrations. An increased tone implies diminished relaxation, leading to smaller amplitude

and higher frequency. The increase in basal tone may be due to an increase in intracellular Ca^{2+}, and a delay in, or inhibition of, removal of intracellular Ca^{2+} to intracellular stores. The release of Ca^{2+} from intracellular stores has been suggested to be the dominant source for endothelin-1-induced increases in internal Ca^{2+} in coronary artery smooth muscle (Wagner-Mann et al., 1991). Pregnancy, associated with several changes in the pattern of myometrial function, could help to explain these findings, including changes in the electrophysiological mechanisms (gap junctions, ionic channels) and biochemical processes (actin/myosin, receptors) controlling myometrial contraction and relaxation (Raemsch and Sommer, 1983; Balducci et al., 1993).

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