Effect of nimodipine on spontaneous, and oxytocin- and carbachol-induced contractions of isolated non-pregnant and pregnant rat myometrium

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Our purpose was to determine the effect of nimodipine on spontaneous, and oxytocin- and carbachol-induced contractions of isolated non-pregnant and pregnant rat myometrium. Amplitude, frequency, duration and integrated area of spontaneous, and oxytocin- and carbachol-induced contractions of non-pregnant and pregnant rat myometrium were compared before and after the addition of nimodipine (IO-[7] to 10[-6]M and 10-6 to 10[-4]M) respectively In myometrial strips isolated from the pregnant rat, the beginning of inhibition of spontaneous, and oxytocin- and carbachol-induced contractions required significantly higher concentration of nimodipine (10[-6] vs. 10[-7]M) and these contractions ceased at significantly higher concentration of nimodipine (10[-4] vs. 10[-6]M). Nimodipine significantly inhibits contractions of myometrial strips isolated from non-pregnant and pregnant rats. Resistance to inhibition in myometrial strips isolated from the pregnant rat can be explained by pregnancy associated changes in the electrophysiological and biochemical factors. [Turk J Med Res 1997; 15(2):43-48]

Key Words: Nimodipine, Myometrium, Rat, Contractions

An increase in intracellular calcium is central to control of contractile activity in myometrium. Calcium influx across the membrane from the extracellular space is partially responsible for the increase in intracellular free calcium. This transfer of extracellular calcium to the intracellular space is via voltage- or receptor-operated channels. The myometrial relaxing properties of calcium channel blockers, a group of heterogeneous agents, are mediated primarily by the voltage-dependent channels. They may also inhibit release of intracellular calcium from sarcolemmal stores and increase calcium efflux from the cell.

Over the last 10 years in obstetrics, calcium channel blockers have been used in pregnancy, primarily in research studies dealing with blood pressure control in patients with preeclampsia (1,2) and for treatment of preterm labor (3). Nifedipine, a first-generation calcium channel blocker, is among the most widely used tocolytic drugs. Three randomized controlled trials have compared nifedipine with (3-adrenergic agonists (4-6). They reported similar efficacy of these two agents with less side effects in the nifedipine-treated group.

The second-generation calcium channel blockers are chiefly nifedipine-like agents, with improved kinetic qualities, such as longer half-lives and greater vascular selectivity with less direct inotropic effects. There is good evidence that dihydropyridine calcium channel blockers (nifedipine (1), nitrendipine (7), isradipine'(8), and nimodipine (9)) do not reduce uteroplacental blood flow in spite of a significant reduction in maternal blood pressure.

Since calcium channel blockers show great promise in treating preterm labor and experimental data about nimodipine are too sparse for its use in preterm labor at this time, this study was performed to systematically examine the effect of nimodipine on spontaneous, oxytocin- and carbachol-induced contractions of myometrium isolated from the non-pregnant and pregnant rat.

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MATERIALS AND METHODS

Tissue preparation. Non-pregnant 200 to 250 g Albino rats (n=5) and timed-pregnant Albino rats (n=5) and were cared for under the guidelines of the Cumhuriyet University at Animal Care Center. 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 in the late-stage pregnant rats were removed and non-uterine tissues were dissected away and discarded. We obtained four full-thickness myometrial muscle strips from each animal. Longitudinal strips were incubated in 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) in jacketed tissue

baths aerated with 95% oxygen and 5% carbon dioxide at 37°C.

Measurement of contractile activity. The uterine strips were suspended at 1 g tension for 30 minutes before the addition of the experimental drugs. Isometric tensions on changes were recorded with a Grass FT03 displacement transducers and displayed on a grass 79E polygraph (Grass, Quincy, Mass., USA). The recorder paper speed was set at 5 mm/sec and calibrated so that 1 cm of vertical displacement represented 1 g of tension.

Three sets of experimental studies were performed. While performing each set of 3 experimental studies, we used three muscle strips obtained from each rat uterus. In the first set of studies, we evaluated the effect of nimodipine on spontaneous contractions in myometrium isolated from non-pregnant rats (n=5) and pregnant rats (n=5). In the second set of studies, we evaluated the effect of nimodipine on oxytocin-induced (submaximal concentration, 2000 mIU) contractions in myometrium isolated from non-pregnant rats (n=5) and pregnant rats (n=5). In the third set of studies, we examined the effect of nimodipine on carbachol-induced (submaximal concentration, 10^{-6}M) contractions in myometrium isolated from non-pregnant rats (n=5) and pregnant rats (n=5).

Drugs. Chemicals used in the current experiments were oxytocin and carbachol purchased from Sigma Chemical (St. Louis, Missouri, USA) and nimodipine obtained from ICN Pharmaceuticals (Costa Mesa, Ca, USA). Drug-containing solutions were prepared immediately before an experiment.

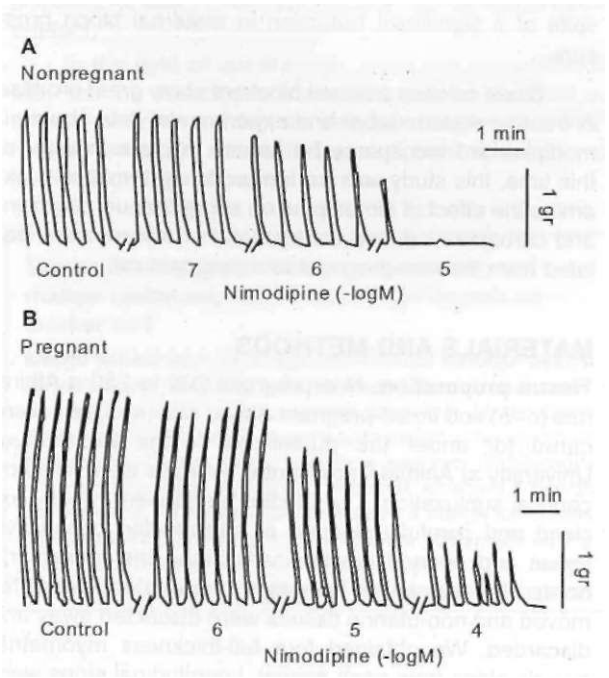

Figure 1.A. Effect of 10^{-7}-10^{-5}M nimodipine on spontaneous contractile activity of myometrium isolated from the non-pregnant rat. **B.** Effect of 10^{-6}-10^{-4}M nimodipine on spontaneous contractile activity of myometrium isolated from the pregnant rat.

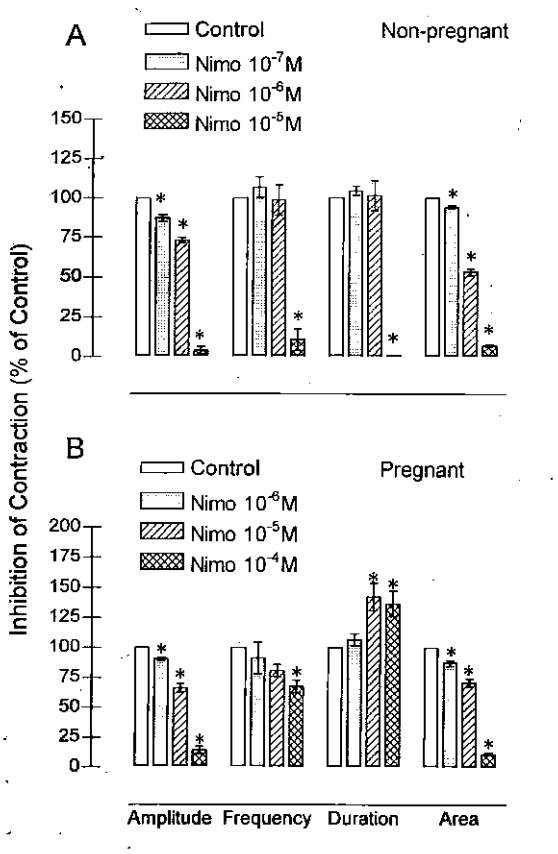

Figure 2.A. Effect of 10^{-7}-10^{-5}M nimodipine (Nimo) on spontaneous contraction of myometrium isolated from non-pregnant rats (n=5). Data (mean ± SE) expressed relative to control (asterisk, $p < 0.05$). **B.** Effect of 10^{-6}-10^{-4}M nimodipine (Nimo) on spontaneous contraction of myometrium isolated from pregnant rats (n=5). Data (mean ± SE) expressed relative to control (asterisk, $p < 0.05$).

Data analysis. The characteristics of the contractions analyzed over 500-second intervals immediately before and after the addition of drugs included frequency (number per 1000 seconds), mean duration (seconds) and amplitude (grams) of each contraction, and integrated area under the contraction curve (representing contractile force over 1000 seconds) measured with a digitized plotter. Data are presented as mean ± SE and were analyzed by analysis of variance and the Newman-Keuls test with $p < 0.05$ considered statistically significant.

RESULTS

Effect of nimodipine on spontaneous contractions of isolated non-pregnant and pregnant rat myometrium.

Nimodipine exposure decreased contractile activity in myometrial strips isolated from the non-pregnant rat (n=5 rats) (Figure 1A and 2A), with the greatest effect on the amplitude and integrated area of contractions. In the am-

plitude and integrated area of contractions, there is statistically significant difference among control and all concentrations of nimodipine (10-^{7}-10"^{5}M). The frequency and duration of contractions changed with increasing concentrations of nimodipine, reaching statistical significance at a concentration of 10-^{5}M.

Nimodipine exposure decreased contractile activity in myometrial strips isolated from the pregnant rat (n=5 rats) (Figure 1B and 2B), with the greatest effect on the amplitude and integrated area of contractions. In the amplitude and integrated area of contractions, there is statistically significant difference among control and all concentrations of nimodipine (10-^{6}-10"^{4}M). The frequency and duration of contractions changed with increasing concentrations of nimodipine, reaching statistical significance at a concentration of 10-^{4}M and concentrations of 10"^{5} and 10-^{4}M respectively.

Effect of nimodipine on oxytocin-induced contractions of isolated non-pregnant and pregnant rat myometrium. Nimodipine exposure decreased contractile activity in myometrial strips isolated from the non-pregnant rat (n=5 rats) (Fig. 3A and 4A), with the significant effect on the amplitude and integrated area of contractions. There is statistically significant difference among control and all concentrations of nimodipine (10-^{7}-10'^{5}M) in the amplitude and integrated area of contractions. The frequency and duration of contractions changed with increasing concentrations of nimodipine, reaching statistical significance at a concentration of 1C^{5}M.

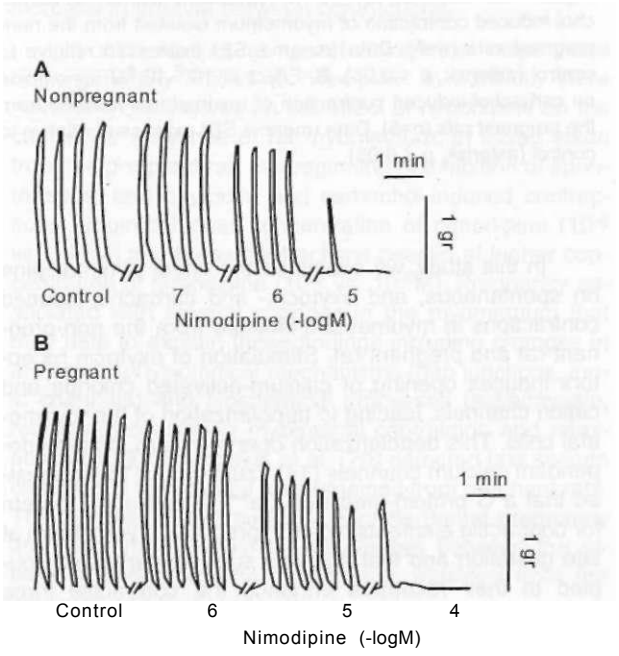

Figure 3.**A.** Effect of 10~^{7}-10"^{5}M nimodipine on oxytocin-induced contractile activity of myometrium isolated from the non-pregnant rat. **B.** Effect of 10~^{6}-10~^{4}M nimodipine on oxytocin-induced contractile activity of myometrium isolated from the pregnant rat.

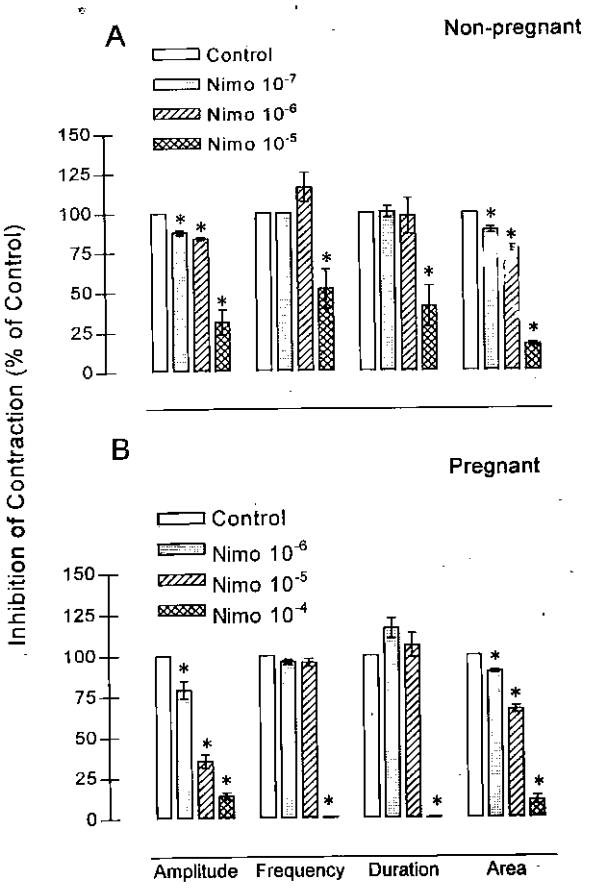

Figure 4.**A.** Effect of 10~^{7}-10"^{5}M nimodipine (Nimo) on oxytocin-induced contraction of myometrium isolated from the non-pregnant rats (n=5). Data (mean ± SE) expressed relative to control (asterisk, $p < 0.05$). **B.** Effect of 10~^{6}-10-4M nimodipine on oxytocin-induced contraction of myometrium isolated from the pregnant rats (n=5). Data (mean ± SE) expressed relative to control (asterisk, $p < 0.05$).

Nimodipine exposure decreased contractile activity in myometrial strips isolated from the pregnant rat (n=5 rats) (Figure 3B and 4B), with the greatest effect on the amplitude and integrated area of contractions. In the amplitude and integrated area of contractions, there is statistically significant difference among control and all concentrations of nimodipine (10~^{6}-1(HM). The frequency and duration of contractions changed with increasing contractions of nimodipine, reaching statistical significance at a concentration of 10~^{4}M.

Effect of nimodipine on carbachol-induced contractions of isolated non-pregnant and pregnant rat myometrium. Nimodipine exposure decreased contractile activity in myometrial strips isolated from the non-pregnant rat (n=5 rats) (Figure 5A and 6A), with the greatest effect on the amplitude and integrated area of contractions. In the amplitude and integrated area of contractions, there is statistically significant difference among control and all concentrations of nimodipine (10"^{7}-10-^{5}M). The frequency and duration of contractions changed with increasing concentrations of nimodipine, reaching statis-

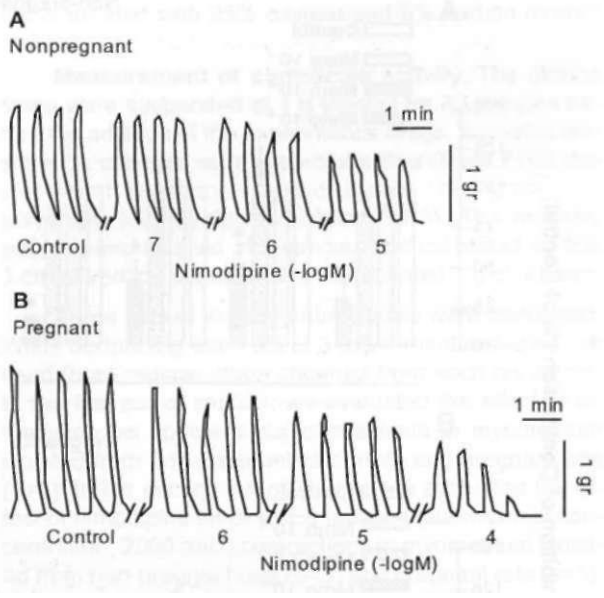

Figure 5.**A.** Effect of 10-⁷-10⁻⁵M nimodipine on carbachol-induced contractile activity of myometrium isolated from the non-pregnant rat. **B.** Effect of 10"⁶-10"⁴M nimodipine on carbachol-induced contractile activity of myometrium isolated from the pregnant rat.

tical significance at a concentration of 10"⁵M and concentrations of 10"⁶ and 10"⁵M respectively.

Nimodipine exposure decreased contractile activity in myometrial strips isolated from the pregnant rat (n=5 rats) (Figure 5B and 6B), with the greatest effect on the amplitude and integrated area of contractions. In the amplitude and integrated area of contractions, there is statistically significant difference among control and all concentrations of nimodipine (10"⁶-10-⁴M). The frequency and duration of contractions changed with increasing concentrations of nimodipine, reaching statistical significance at a concentration of 10"⁴M and concentrations of 10"⁵ and K H M respectively.

DISCUSSION

The intracellular concentration of C a⁺⁺ is thought to play an essential role in regulation of the contraction-relaxation cycle in uterine smooth muscle cells. An increase in the intracellular C a⁺⁺ in rat and human myometrial smooth muscle cells forms a Ca⁺⁺-calmodulin complex and activates myosin light chain kinase to phosphorylate myosin light chain and initiates myometrial cell contractions (10,11). The degree of activation of smooth muscle contractile proteins is related directly to changes in the cytoplasmic calcium concentration. The concentration of free calcium in the cytoplasm can bj increased by entry of calcium through either voltage-dependent or receptor-operated channels. Calcium also may be released from intracellular binding sites, such as the internal surface of the cell membrane as well as the sarcoplasmic reticulum and mitochondria (12,13).

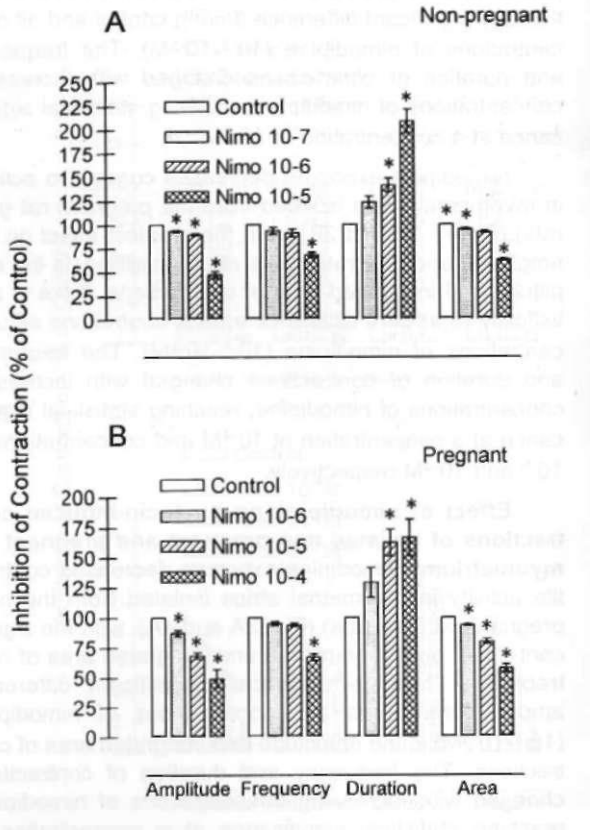

Figure 6.A. Effect of 10"⁷-10-⁵M nimodipine (Nimo) on carbachol-induced contraction of myometrium isolated from the non-pregnant rats (n=5). Data (mean ± SE) expressed relative to control (asterisk, $p < 0.05$). **B.** Effect of 10"⁶-10-⁴M nimodipine on carbachol-induced contraction of myometrium isolated from the pregnant rats (n=5). Data (mean ± SE) expressed relative to control (asterisk, $p < 0.05$).

In this study, we examined the effect of nimodipine on spontaneous, and oxytocin- and carbachol-induced contractions in myometrium isolated from the non-pregnant rat and pregnant rat. Stimulation of oxytocin receptors induces opening of calcium-activated chloride and cation channels, leading to depolarization of the myometrial cells. This depolarization opens, in turn, voltage-dependent calcium channels (14). Izumi et al. (15) suggested that a G protein-mediated C a⁺⁺ sensitization system for contractile elements exists in pregnant myometrium at late gestation and that agonists such as carbachol coupled to their receptors enhance the contractile force through this system and thereby augment expelling forces that occur during labor and delivery of the fetuses.

The calcium channel blockers are a heterogeneous group of compounds that act mainly by inhibiting the influx of calcium ions through the cell membrane. The main site of action of the calcium channel blockers is believed to be the voltage-dependent channels where inhibition of

the influx of extracellular calcium results in uncoupling of excitation and contraction. Inhibition of calcium influx may not only directly decrease the concentration of cytoplasmic calcium, but also cause a decrease in calcium release from intracellular stores. In addition, other relaxant effects of these drugs at later steps in the excitation-contraction coupling process have been suggested. Various types of calcium channel blockers exist that are classified based on the core chemical structure. Although they all inhibit calcium influx, these compounds have a variable spectrum of actions. The 1,4 dihydropyridine-type calcium channel blockers, nifedipine and nicardipine, are more selective and potent inhibitors of uterine contractions. Nifedipine is the prototype calcium channel blocker used in clinical trials for tocolysis (16). In vitro studies performed on human myometrial tissue from non-pregnant and pregnant women have shown that nifedipine is a potent inhibitor of myometrial contractions (17). Nifedipine inhibited spontaneous contractility and contractions elicited by vasopressin and potassium chloride in myometrial strips obtained from women undergoing hysterectomy. In strips from pregnant myometrium, nifedipine inhibited spontaneous contractility and contraction induced by oxytocin and prostaglandins. Nifedipine was found to be more potent in relaxing potassium chloride-induced contractions in term pregnant myometrium than in non-pregnant myometrium. Using myometrial tissue from women undergoing Cesarean Section throughout the third trimester, Bird et al. (3) found that nifedipine produced a dose-related decrease in oxytocin-induced contraction strength without a consistent increase in interval between contractions.

In vitro studies using rat myometrial strips have been generally limited to nifedipine. In addition, there have been no studies on the effect of nimodipine on the contractile response of rat myometrium. In tissue taken from the pregnant rat, the beginning of inhibition of spontaneous, and oxytocin- and carbachol-induced contractions required higher concentration of nimodipine (10'^{6} vs. 10-7M) and these contractions ceased at higher concentration of nimodipine (1(H vs. 10"^{5}M). Pregnancy associated with several changes in the myometrium that may 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 (18,19). Sperelakis et al. (20) detected fast sodium channels in myometrial cells obtained from pregnant rats. The increase in fast sodium channels during pregnancy and labor may explain the differences between the effects of nimodipine on myometrium obtained from the pregnant and non-pregnant rat.

In conclusion, nimodipine inhibits myometrial contractions of the non-pregnant and pregnant rat. With confirmation of these effects in a clinical study of patients with preterm labor, nimodipine, a second-generation calcium channel blocker, can be used as an alternative to nifedipine.

Nimodipinin gebe olmayan ve gebe olan İzole rat myometriumunun spontan ve oksitosin ve karbakol ile uyarılmış kontraksiyonlarına etkisi

Bu çalışma nimodipinin gebe olmayan ve gebe olan izole rat myometriumunun spontan ve oksitosin ve karbakol ile uyarılmış kontraksiyonlarına etkisini incelemek amacıyla yapıldı. Gebe olmayan ve gebe olan rat izole myometriumunda sırasıyla 10~^{7}-10~^{5}M ve 10^{6}-10^{4}M nimodipin eklenmesinden önce ve sonraki spontan; oksitosin ve karbakol ile uyarılmış kontraksiyonların amplitüd, sıklık, süre ve eğri altındaki alan parametreleri karşılaştırıldı. Gebe ratlardan elde edilen myometrial dokuda, spontan ve oksitosin ve karbakol ile uyarılmış kontraksiyonlarda inhibisyonun başlaması anlamlı olarak daha yüksek konsantrasyonda nimodipin (10^{6}M yerine 10^{6}) gerektirdi ve bu kontraksiyonlar anlamlı olarak daha yüksek konsantrasyonda nimodipin (10^{6}M yerine 10^{4}) ile tamamen durdu. Nimodipin gebe olmayan ve gebe olan ratlardan alınan myometrial doku örneklerinin konfeksiyonlarını anlamlı olarak inhibe etmektedir. Gebe ratlardan alınan doku örneklerinde inhibisyonun gebe olmayan doku örneklerine göre daha zor olması gebeliğe bağlı olarak myometriumda oluşan elektrofizyolojik ve biyokimyasal değişikliklere bağlanabilir. [T Klin Araştırma 1997; 15(2):43-48]

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