The Effect of Omeprazole in Myometrial Smooth Muscle Isolated from Nonpregnant and Pregnant Rats

OMEPRAZOLÜN GEBE VE GEBE OLMAYAN SIÇANLARDAN İZOLE EDİLEN MYOMETRİAL DÜZ KASLARDAKİ ETKİSİ

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Summary

Objective: The present experiments were designed to investigate the effect of omeprazole, a H^+-K^+ ATPase inhibitor, on myometrial smooth muscle isolated from nonpregnant and pregnant rats.

Material and Methods: Isolated myometrial strips were obtained from pregnant (n=10) and nonpregnant (n=10) rats and were mounted in organ baths for recordings of isometric tensions. The effect of increasing concentrations of omeprazole (10^{-5}-10^{-3} M) on spontaneous myometrial contractions and on myometrial smooth muscles precontracted with 8 mM Ca^{2+} in Ca^{2+}-free medium were recorded.

Results: Omeprazole (10^{-5}-10^{-3} M) decreased the amplitude and frequency of spontaneous contractions time- and concentration-dependently in myometrial smooth muscle isolated from nonpregnant and pregnant rats. Addition of indomethacin (3.10^{-6} M), N^G-nitro-L-arginine methyl ester (L-NAME) (3.10^{-5} M), sodium acetate (7.5 mM) and ammonium chloride (0.5 mM) in to the organ baths 20 min before did not change the responses to omeprazole in all myometrial smooth muscles. Omeprazole also relaxed time- and concentration-dependently the myometrial smooth muscles precontracted with 8 mM Ca^{2+} in Ca^{2+}-free medium, but the relaxation response of nonpregnant rat myometrial smooth muscles was significantly higher than that of pregnant rat myometrial smooth muscles in all concentrations.

Conclusions: Omeprazole inhibites spontaneous contractions of myometrial smooth muscle in pregnant and nonpregnant rats. The inhibition of spontaneous contractions and the relaxation of myometrial smooth muscle precontracted with Ca^{2+} by omeprazole with Ca^{2+} myometrial smooth muscle by omeprazole is probably mediated by the blockade of calcium channels.

Key Words: Omeprazole, H^+-K^+-ATPase, Uterus, Smooth muscle, Pregnancy

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Özet

Amaç: Bu deneysel çalışma bir H^+-K^+ ATPaz inhibitörü olan omeprazolün gebe ve gebe olmayan sıçanlardan izole edilen myometrial düz kas şeritleri üzerindeki etkisini araştırmak için planlandı.

Gereç ve Yöntem: 10 adet gebe ve 10 adet gebe olmayan sıçandan izole edilen myometrial şeritler izometrik kasılmaları kaydetmek için organ banyolarına yerleştirildi. Spontan myometrial kontraksiyonlar ve Ca^{2+} olmayan bir ortamda 8mM Ca^{2+} ile kastırılmış myometrial düz kaslar üzerinde omeprazol'ün artan konsantrasyonlarının (10 - 10^{-3}) etkisi kaydedildi.

Bulgular: Gebe ve gebe-olmayan sıçanlardan izole edilen myometrial düz kaslarda, omeprazol zamana ve konsantrasyona-bağımlı olarak spontan myometrial kontraksiyonların amplitüd ve frekansını azalttı. Myometrial düz kasların bulunduğu organ banyolarına omeprazol uygulamadan 20 dak. önce indometazin (3.10^{-6} M), N_G-nitro-L-arjinin metil ester (L-NAME) (3.10^{-5} M), sodyum asetat (7.5 mM) ve amonyum klorid (0.5 mM) ilave edilmesi omeprazolün gevşeme yanıtlarını değiştirmedi. Ayrıca; omeprazol Ca^{2+} olmayan ortamda 8mM Ca^{2+} ile kastırılmış myometrial düz kaslarda zamana ve konsantrasyona-bağımlı olarak gevşeme yanıtlarına neden oldu, ancak gebe olmayan sıçanlardaki gevşeme yanıtları, gebe sıçanlara ait gevşeme yanıtlarından tüm konsantrasyonlarda anlamlı olarak daha fazlaydı ($p<0.05$).

Sonuç: Omeprazol gebe ve gebe olmayan sıçanların myometrial düz kaslarındaki spontan kontraksiyonları inhibe etmektedir. Spontan myometrial kontraksiyonların ve Ca^{+} ile kastırılmış myometrial düz kasların omeprazol ile inhibisyonu muhtemelen myometrial düz kastaki kalsiyum kanallarının blokajına bağlıdır.

Anahtar Kelimeler:

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The tT-K* ATPase is an active transport mechanism that mediates K^+ uptake into cells in exchange for H^+efflux (1). The HT-K* ATPase was first described in the gastric parietal cell where it mediates gastric acid secretion into the stomach lumen (2). The H^+-K^+-ATPase is a family of the P-type ATPases that are a phosphorylating class of ion transport ATPases (3). The ET-K"'-ATPases are further divided into isoforms of gastric (4), colonic (5), and possibly bladder (6). H^+-K^+-ATPase isoforms are present in organs other than the stomach, colon, and bladder, including the kidney (7), vascular smooth muscle (3), airway smooth muscle (8), corpus cavernosum smooth muscle (9) and human polymorphonuclear leucocytes (10). The colonic H^+-K^+-ATPase has been localized to the distal colon surface epithelial cells and myometrial tissue (5). In the uterus, H^+-K^+-ATPase is present in the region of the myometrium between the inner and midmuscular zone that is very rich in vascular supply and nerve cells. This finding suggests that the H^+-K^+-ATPase may not be involved in the control of pH and potassium concentration of the uterine fluid but rather in distinct functions of vascular and/or nerve cells (5).

The H^+-K^+ ATPase inhibitors (proton pump inhibitors), omeprazole, lansoprazole, and pantoprazole bind irreversibly to the proton pump itself, directly inhibiting hydrogen ion exchange and inhibiting acid secretion in response to all stimulatory agents (11). Omeprazole and SCH 28080 (a H^+-K^+ ATPase inhibitor) induce concentration-dependent relaxation of guinea pig trachea with spontaneous tone and precontracted with carbachol or histamine. SCH 28080 also induces concentration-dependent relaxation of human bronchi precontracted with histamine (8). Leminoprazole relaxed the isolated rat aorta contracted by phenylephrine, KC1 and Ca^{2+} in Ca^{2+}-free medium (12). These findings demonstrate that H^+-K^+ ATPase inhibitors cause relaxation of airway and vascular smooth muscles in vitro.

Our purpose was to determine the effect of omeprazole on spontaneous contractions and Ca^{2+}-induced contractions in myometrial smooth muscle isolated from nonpregnant and pregnant rats.

Materials and Methods

Nonpregnant (n=10) and timed-pregnant (at 21-22 days of pregnancy, n=10) Albino rats (200-250 g) were cared for under the guidelines of the Cumhuriyet University, 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 three full-thickness myometrial smooth muscle strips from each animal. Longitudinal smooth muscle strips were incubated in modified Krebs' solution (composition in mmol l^{-1}: 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 (pH = 7.4).

The uterine smooth muscle strips were suspended at 1-g tension for 30 minutes before the addition of the experimental drugs. Isometric tensions on changes were recorded with Grass FT03 displacement transducers and displayed on a Grass 79E polygraph (Grass, Quincy, Mass., USA). The recorder paper speed was set at 5 mm min^{-1} and calibrated so that 1-cm of vertical displacement represented 1-g of tension. 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. After establishment of spontaneous myometrial contractions in the modified Krebs' solution, the effect of increasing concentrations of omeprazole on spontaneous contractions and contraction of myometrial smooth muscles precontracted with 8 mM Ca^{2+} in Ca^{2+}-free medium were recorded. Potassium ion was added in stead of Ca^{2+} into Ca^{2+}-free solution.

Four sets of experimental studies were performed in myometrial smooth muscle isolated from nonpregnant (n=10) and pregnant (n=10) rats. In the first set of studies, we evaluated the effect of omeprazole (10^{-5} to 10^{-3} M) on spontaneous

contractions of myometrial smooth muscle isolated from nonpregnant and pregnant rats. In the second sets of studies, we evaluated the effect of omeprazole on myometrial smooth muscle pretreated with indomethacin (3.10^{-6} M) and L-NAME (3.10^{-5} M) 20 min before omeprazole administration. In the third sets of studies, we evaluated the effect of omeprazole on myometrial smooth muscle pretreated with sodium acetate (7.5 mM) and ammonium chloride (7.5 mM) 20 min before omeprazole administration. In the fourth set of studies, we evaluated the effect of omeprazole in myometrial smooth muscle precontracted with 8 mM Ca^{2+} in Ca^{2+}-free medium. Ca^{2+}-free medium was prepared by omitting **CaCl2** from the solution. In Ca^{2+}-free medium experiments, tissues were incubated in the medium for 20 min before the application of 8 mM Ca^{2+}.

Chemicals used in the current experiment were omeprazole from Astra AB (Sodertalje, Sweden), indomethacin and L-NAME purchased from Sigma Chemical (St. Louis, Missouri, USA). All drugs were dissolved in distilled water except for indomethacin dissolved in 1% 1^CC^, omeprazole dissolved in polyethylene glycol 400. The 1% Na_2C0_3 and polyethylene glycol 400 had no effect *per se* on spontaneous contractile activity of myometrial smooth muscle. All drugs were freshly prepared on the day of the experiments.

In myometrial smooth muscle, while obtaining concentration-response curves of omeprazole, increasing concentrations of omeprazole added to the organ baths. After the relaxation responses of omeprazole reached a stabile plateau, we added higher concentrations of omeprazole to the organ baths. The characteristics of contractions (included amplitude and frequency) analyzed as a percentage of spontaneous contractions after the addition of drugs. The omeprazole relaxation responses on myometrial smooth muscle precontracted by 8 mM Ca^{2+} in Ca^{2+} -free medium was estimated as a change of maximum contraction as a percentage of maximal contraction elicited by 8 mM Ca^{2+} for each strip. Data are presented as means ± SE and were analyzed using one-way ANOVA with repeated measures and ANOVA with Newman-Keuls test when appropriate. A p value of < 0.05 was considered significant.

Results

Omeprazole exposure (10^{-5}-10^{-3} M) in a time- and concentration-dependent manner decreased spontaneous contractile activity in myometrial smooth muscle isolated from the nonpregnant (n=10) and pregnant (n=10) rats (Figure 1). In myometrial smooth muscle isolated from the nonpregnant rats, the amplitude and frequency of spontaneous contractions decreased time- and concentration-dependently by omeprazole, reaching statistical significance at 10^{-4} mol/1 ($p < 0.05$) (Figure 2, 3). In myometrial smooth muscle isolated from the pregnant rats, the amplitude and frequency of spontaneous contractions decreased time- and concentration-dependently by omeprazole, reaching statistical significance at 3.10^{-4}M ($p < 0.05$) (Figure 2, 3). No significant difference was found between omeprazole-induced relaxant effect on spontaneous contractions of myometrial smooth muscles isolated from nonpregnant and pregnant rats. ($p > 0.05$) (Figure 2, 3).

To investigate whether relaxation induced by omeprazole is due to an interaction with the cyclooxygenase or the nitric oxide pathways, myometrial strips were pretreated with indomethacin and L-NAME, respectively. Addition of indomethacin (3.10^{-6} M) and L-NAME (3.10^{-5} M) into the organ baths for 20 min before did not change the amplitude and frequency of omeprazole-induced relaxation responses significantly (Figure 4, 5).

In order to determine whether alkalinization or acidification could mediate relaxant responses to omeprazole, the effect of sodium acetate and ammonium chloride were examined on myometrial strips isolated from nonpregnant and pregnant rats. Prerreatment (20 min.) of myometrial strips with sodium acetate (7.5 mM) and ammonium chloride (7.5 mM) did not significantly alter the relaxant response to omeprazole (Data not shown)

Our previous studies demonstrated that the nimodipine and isradipine, L-type Ca^{2+}channel

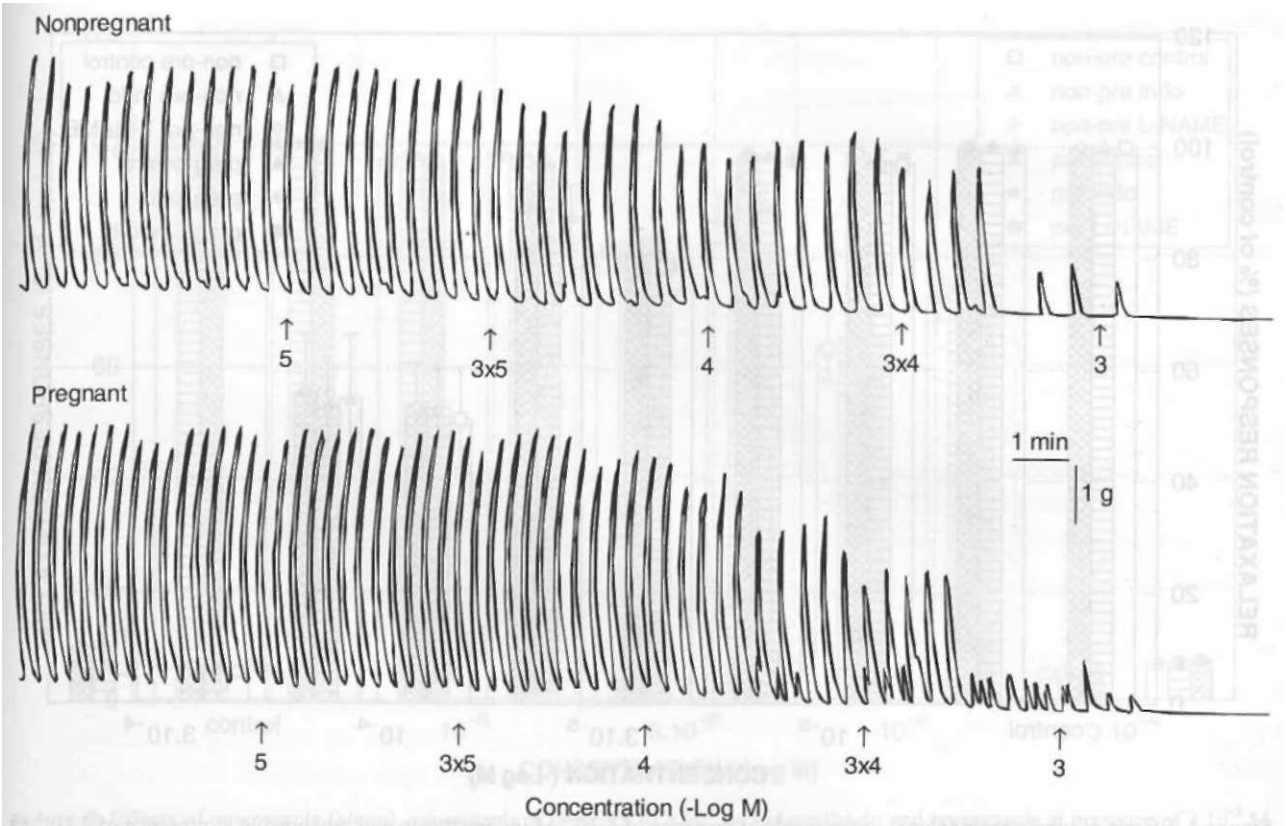


Figure 1. Original response to increasing concentrations of omeprazole (10^{-5}-10^{-3} M) on spontaneous contractions of myometrial strips isolated from nonpregnant and pregnant rats.

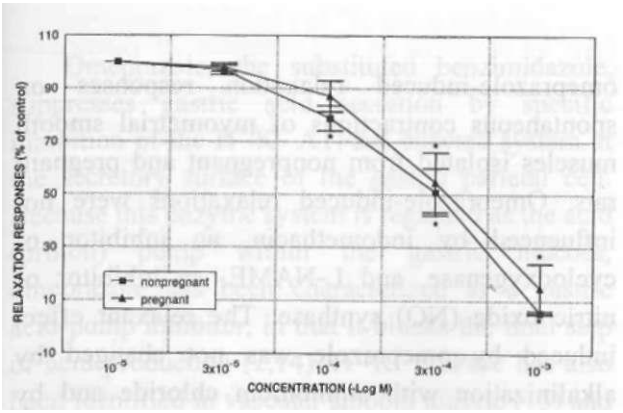


Figure 2. Effect of omeprazole (10"-10"' M) on the amplitude of spontaneous contractions of myometrial strips isolated from non-pregnant and pregnant rats. Values represent mean ± SE of ten preparations. *Significantly different from the control (p<0.05)

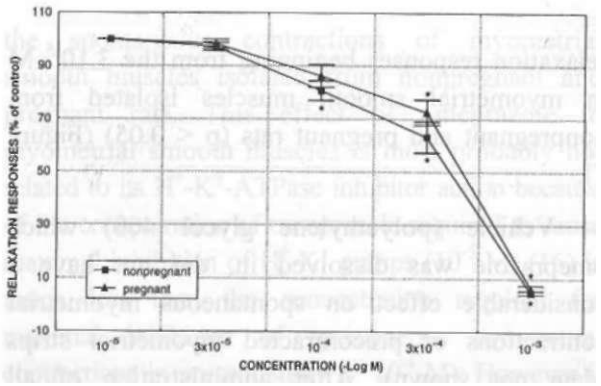


Figure 3. Effect of omeprazole (lO'MfJ"' M) on the frequency of spontaneous contractions of myometrial strips isolated from non-pregnant and pregnant rats. Values represent mean ± SE of ten preparations. * Significantly different from the control (p<0.05)

blockers relaxed spontaneous or oxytocin- and carbachol- stimulated contractions of rat myometrial smooth muscle (13). In this study, omeprazole (10"'-10"' M) time- and concentration-dependently produced relaxation responses in myometrial smooth muscle precontracted with 8 mM Ca^{2+} in Ca^{2+}-free medium, reaching statistical significance at 3.10"' M both in nonpregnant and pregnant rats ($p < 0.05$) (Figure 6, 7). There was a significant difference between omeprazole-induced

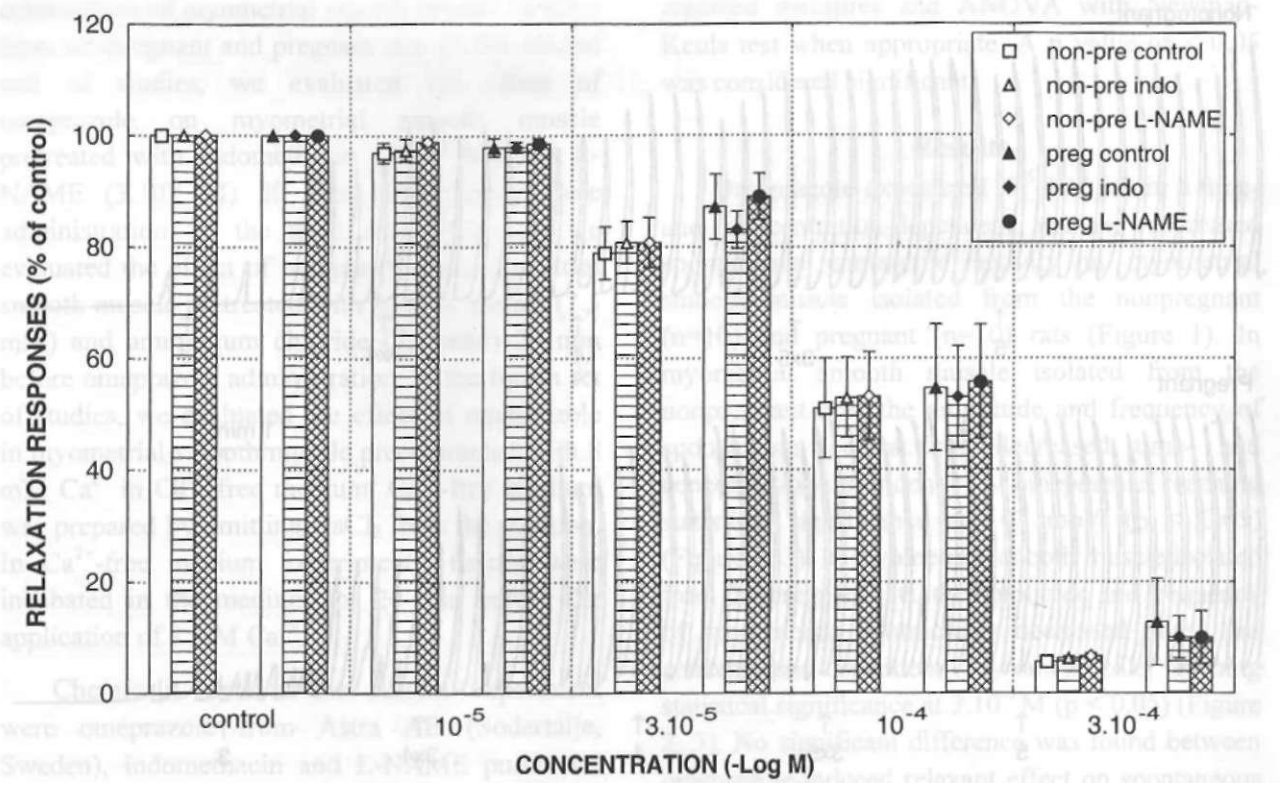


Figure 4. Effects of omeprazole (alone), omeprazole at presence of 3.10^{-6} M indomethacin and omeprazole at presence of 3.10^{-5} M L-NAME, on the amplitude of spontaneous contractions of myometrial smooth muscles isolated from pregnant and nonpregnant rats. Indomethacin (3.10^{-6} M) and L-NAME (3.10^{-5} M) were added to the bath 20 min before the addition of omeprazole. Data are expressed as the means ± SE of 10 experiments.

relaxation responses beginning from the 3.10^{-5} M in myometrial smooth muscles isolated from nonpregnant and pregnant rats ($p < 0.05$) (Figure 7).

Vehicle (polyethylene glycol 400) which omeprazole was dissolved in, did not have a considerable effect on spontaneous myometrial contractions or precontracted myometrial strips (data not shown). After administration of all concentrations of drugs, we washed out the myometrial strips and obtained initial levels of spontaneous contractions (data not shown).

Discussion

These results demonstrate that omeprazole causes the relaxation response in myometrial smooth muscle of nonpregnant and pregnant rats in a time- and concentration-dependent manner. No significant difference was found between omeprazole-induced relaxation responses on spontaneous contractions of myometrial smooth muscles isolated from nonpregnant and pregnant rats. Omeprazole-induced relaxations were not influenced by indomethacin, an inhibitor of cyclooxygenase, and L-NAME, an inhibitor of nitric oxide (NO) synthase. The relaxant effect induced by omeprazole was not changed by alkalinization with ammonium chloride and by acidification with sodium acetate. Omeprazole brought about relaxation time- and concentration-dependently in myometrial smooth muscles precontracted by Ca^{2+} in Ca^{2+} -free medium. There were significant differences between omeprazole-induced relaxation responses in myometrial smooth muscle precontracted by 8 mM Ca^{2+} in Ca^{2+} -free medium isolated from nonpregnant rats compared to that of pregnant rats.

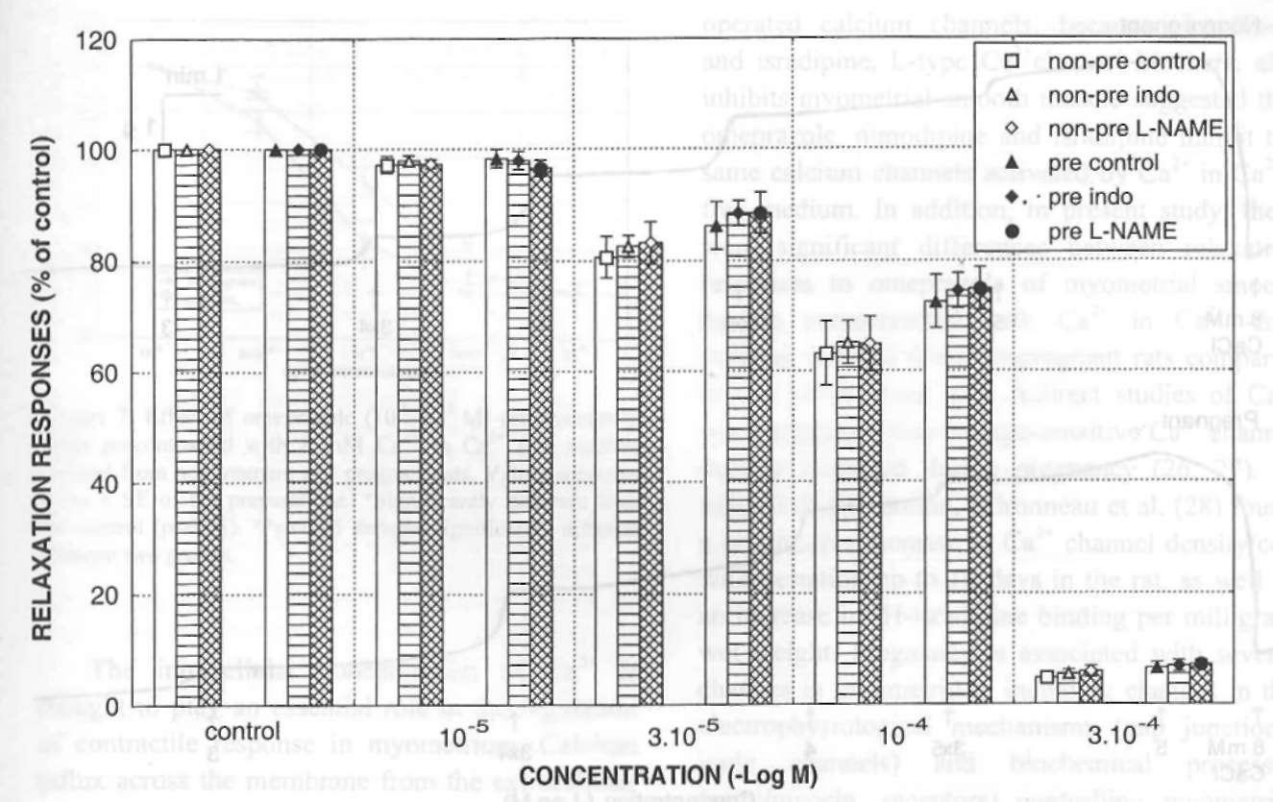
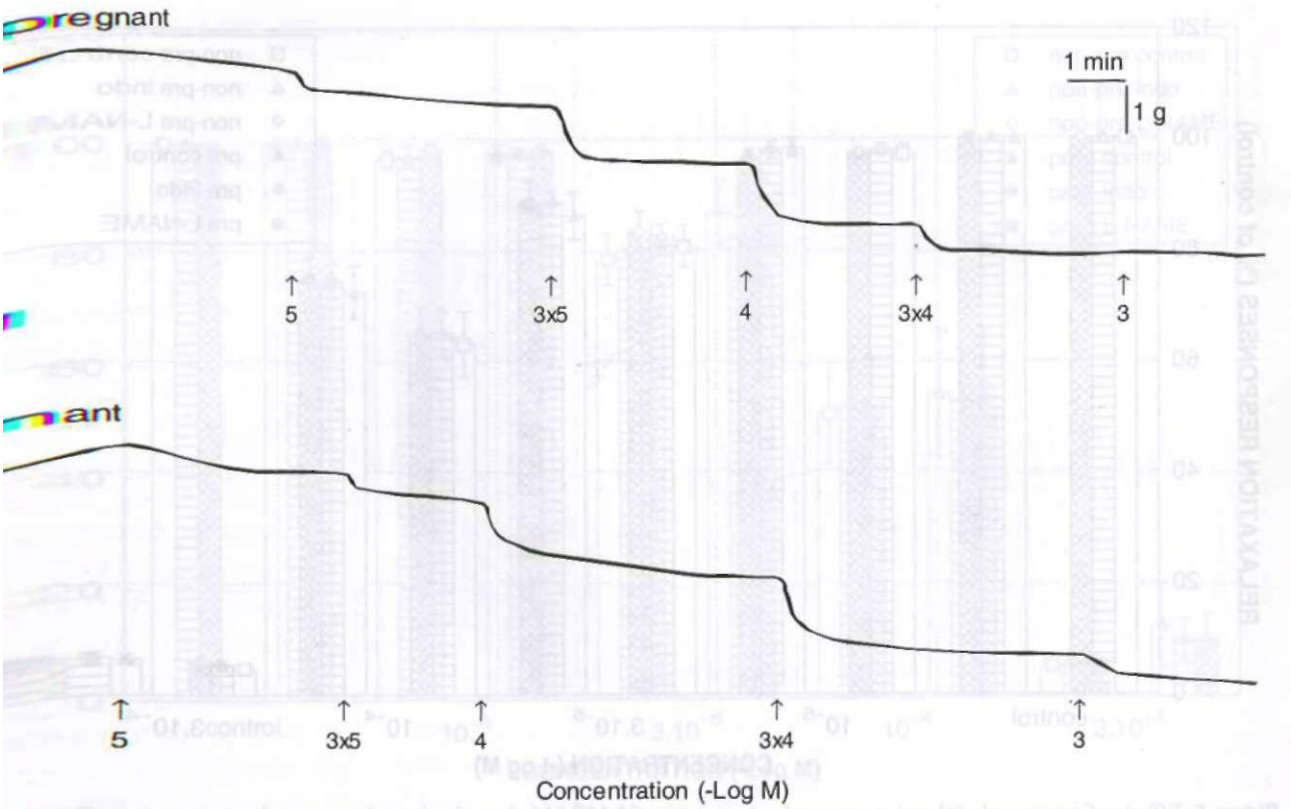


Figure 5. Effects of omeprazole (alone), omeprazole at presence of 3.10^{-6} M indomethacin and omeprazole at presence of 3.10^{-5} M L-NAME, on the frequency of spontaneous contractions of myometrial smooth muscles isolated from pregnant and nonpregnant rats. Indomethacin (3.10^{-6} M) and L-NAME (3.10^{-5} M) were added to the bath 20 min before the addition of omeprazole. Data are expressed as the means ± SE of 10 experiments.

Omeprazole, the substituted benzimidazole, suppresses gastric acid secretion by specific inhibition of the H^+/K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell. Because this enzyme system is regarded as the acid (proton) pump within the gastric mucosa, omeprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production (2,14). H^+-K^+ATPase has also been identified in vascular smooth muscle (15) and leminoprazole (a H^+-K^+ ATPase inhibitor) concentration-dependently inhibited the rat aorta contracted by phenylephrine and KC1 (12). Four H^+-K^+ ATPase inhibitors, SCH 28080, SK&F 96067, omeprazole and NC-1300-B, have also been found to inhibit the guinea pig trachea with spontaneous tone concentration-dependently (8), suggesting that benzimidazole derivatives may indeed inhibit smooth muscle tone. In this study, omeprazole concentration-dependently inhibited the spontaneous contractions of myometrial smooth muscles isolated from nonpregnant and pregnant rats. This effect of omeprazole in myometrial smooth muscles is most probably not related to its H^+-K^+-ATPase inhibitor action because of the concentration of omeprazole required to cause maximal inhibition of H^+-K^+ pumps (10^{-5} M) (16) is much less than the concentration required for maximal inhibition of spontaneous myometrial contractions in present study (> 10^{-4} M). However it should be kept in mind that the highest concentrations of proton pumps inhibitors have been reported to affect other ion-motive ATPases like Na^+-K^+-ATPase and vacuolar rf-ATPase (17). Omeprazole inhibits H^+-K^+-ATPase through a covalent modification of the enzyme. Omeprazole itself is not active, but requires FT mediated conversion to a sulfonamide derivative, which then reacts with sulfhydryl groups on specific cysteine residues of the alpha subunit (15). The requirement of



. Effects of cumulative concentrations of omeprazole on myometrial strips precontracted with 8 mM Ca^{2+} in Ca^{2+}-free isolated from nonpregnant and pregnant rats.

zole to be converted to an active metabolite is nt with the slow time course of omeprazole-l relaxation observed in this study.

staglandin E and $F_{2\alpha}$, arachidonic acid lites, are examples of main endogenous c agents and inhibit the action of adenyl subsequently inhibiting formation of and causing uterine contractions (18). We trated that indomethacin, when used at rations known to be experimentally and tically effective at inhibiting genase in the myometrium, had no t effect on omeprazole-induced relaxation s. We conclude that activation of genase is not required for omeprazole-inhibition in nonpregnant and pregnant rat rium.

has a relaxant effect on myometrium (19, is produced within the female genital tract regnancy and a reduction in NO synthesis may be involved in the initiation of parturition (21). The administration of NO donors may be useful in inhibiting uterine contractions in situations where such activity is unwanted, e.g., in preterm labor (22). NO production is inhibited by L-NAME. In this study, addition of L-NAME in to the organ baths for 20 min before did not change omeprazole-induced relaxation responses in myometrial smooth muscle isolated from nonpregnant and pregnant rats. These findings suggest that omeprazole-induced relaxation responses in nonpregnant and pregnant rat myometrium are not mediated by NO. It is well known that a number of other smooth muscles are relaxed by a decrease in intracellular pH (23). However this possibility seems unlikely, since in this study the relaxant effect induced by omeprazole in myometrial smooth muscles was not changed by alkalinization with ammonium chloride and by acidification with sodium acetate.

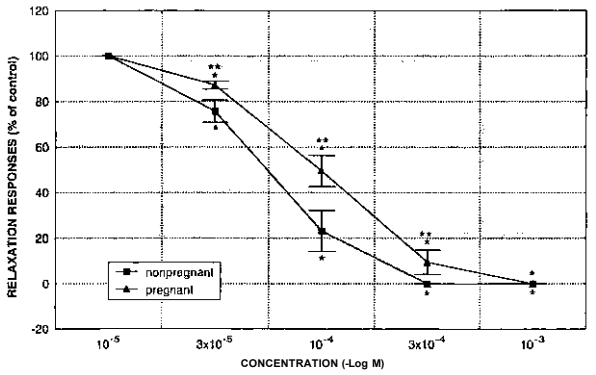


Figure 7. Effect of omeprazole (10^{-5}-10^{-3} M) on myometrial strips precontracted with 8 mM Ca^{2+} in Ca^{2+} free medium isolated from nonpregnant and pregnant rats. Values represent mean ± SE of ten preparations. * Significantly different from the control ($p<0.05$). **$p<0.05$ denotes significantly different between two groups.

The intracellular concentration of Ca is thought to play an essential role in the regulation of contractile response in myometrium. Calcium influx across the membrane from the extracellular space is partially responsible for the increase in intracellular free calcium, which stimulates activation of the myosin light chain kinase and initiates myometrial cell contractions (24). The concentration of free Ca^{2+} in the cytoplasm can be increased by entry of calcium through either voltage dependent or receptor-operated channels (25). Okabe et al. (12) suggested that the vasoinhibitory effect of leminoprazole, a proton pump inhibitor, in rat aortic rings is due to inhibition of voltage operated Ca^{2+} channels. We previously demonstrated that the nimodipine and isradipine, L-type Ca^{2+}channel blockers, inhibited the spontaneous and oxytocin- and carbachol-stimulated contractions of rat myometrium in a concentration-dependent manner (13). It is well known that Ca^{2+}-induced contractions in the Ca^{2+}-free medium are due to an increase in Ca^{2+} influx through voltage-operated channels. In this study, we found that omeprazole time- and concentration dependently inhibited myometrial smooth muscle precontracted with 8 mM Ca^{2+} in Ca^{2+} -free medium. The ability of omeprazole to inhibit myometrial smooth muscle precontracted with Ca^{2+}indicates that omeprazole may inhibit voltage-operated calcium channels, because nimodipine and isradipine, L-type Ca^{2+}channel blockers, also inhibits myometrial smooth muscle suggested that omeprazole, nimodipine and isradipine inhibit the same calcium channels activated by Ca^{2+} in Ca^{2+}-free medium. In addition, in present study, there were significant differences between relaxation responses to omeprazole of myometrial smooth muscle precontracted with Ca^{2+} in Ca^{2+} -free medium isolated from nonpregnant rats compared to that of pregnant rats. Indirect studies of Ca^{2+} entry suggested that voltage-sensitive Ca^{2+} channel density increased during pregnancy (26, 27). In support of this notion, Mironneau et al. (28) found a progressive increase in Ca^{2+} channel density/cell with gestation up to 18 days in the rat, as well as an increase in ^{3}H-isradipine binding per milligram wet weight. Pregnancy is associated with several changes in myometrium, including changes in the electrophysiological mechanisms (gap junctions, ionic channels) and biochemical processes (actin/myosin, receptors) controlling myometrial contraction and relaxation (29-31). It is possible that hormonal status can modify the properties of calcium channels with which the omeprazole interact. This may explain the difference in omeprazole responses of myometrial smooth muscles precontracted with Ca^{2+} obtained from nonpregnant and pregnant rat. We previously showed that omeprazole inhibited contractions of isolated human myometrial smooth muscle. Effect of omeprazole were similar both in human and rat myometrium (32).

The present study indicates that omeprazole (at highest concentrations) inhibited the spontaneous contractions and Ca^{2+}-induced contractions in myometrial smooth muscle isolated from nonpregnant and pregnant rats in a time- and concentration dependent manner. Inhibitor effect of omeprazole is not related to cyclooxygenase products, NO and pH changes, but it may be due to the difference in the function of voltage-operated Ca^{2+} channels. Further work is needed to determine the cellular mechanisms of action by which omeprazole acts on myometrial smooth muscle.