EVIDENCE OF RELAXANT EFFECT OF OMEPRAZOLE IN RABBIT CORPUS CAVERNOSUM IN VITRO

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Summary

The present experiments were designed to investigate the effects of omeprazole, a H⁺-K⁺ ATPase inhibitor, on corporal smooth muscle tone in vitro. All spontaneous contractile activity in the corpus cavernosum was blocked following omeprazole (0.1 mM-1 mM) administration. However atropine (1 µM), Nω-nitro L-arginine methyl ester (L-NAME, 30 µM) or indomethacin (10 µM) did not affect the spontaneous contraction. Omeprazole (10 µM-1 mM) concentration-dependently induced relaxation in corporal smooth muscle precontracted with 10 µM phenylephrine or 80 mM KCl. Pretreatment of corporal tissue with L-NAME (30 µM), indomethacin (10 µM), ammonium chloride (7.5 mM), sodium acetate (7.5 mM), tetroethyl ammonium chloride (0.5 mM) or glibenclamide (1 µM) had no effect on the omeprazole induced relaxant responses. Nimodipine, an L-type Ca⁺⁺ channel blocker, relaxed corporal strips precontracted with 80 mM KCl. Collectively, these results indicate that the inhibition of spontaneous contraction and the relaxation of precontracted corporal smooth muscle by omeprazole is probably mediated by the blockade of calcium channels. Further work is needed to determine the cellular mechanism(s) of action by which omeprazole acts on corpus cavernosum smooth muscle.

Key Words: corpus cavernosum, H⁺-K⁺ ATPase inhibitors, omeprazole, smooth muscle
The H⁺-K⁺ ATPase was first described in the gastric parietal cell where it mediates gastric acid secretion into the stomach lumen (1). Similar pumps have been identified in other specialised epithelia (2). Recent studies have suggested that nonepithelial cells may also possess H⁺-K⁺ ATPase activity. H⁺-K⁺ ATPase inhibitors cause a reduction in potassium (K⁺) uptake, K⁺ content and intracellular pH in cultured vascular smooth muscle cells as well as in intact vascular tissue (3). The presence of a H⁺-K⁺ATPase in the plasma membrane of smooth muscle cells may have implications for the regulation of contractile function because, changes in intracellular pH are known to modulate smooth muscle tone (4) and the K⁺ concentration gradient is one of the major determinants of the membrane potential.

The aim of the present study, was to investigate the role of omeprazole in the regulation of corpus cavernosum smooth muscle contractile function in vitro.

Methods

Experiments were performed on ten mature male albino rabbits weighing 2.5 to 3 kg. The animals were anaesthetised with sodium pentobarbital (50 mg/kg, iv) and their penises were surgically removed. The corpus cavernosum tissue was carefully dissected free from the surrounding tunica albuginea and strips of corpus cavernosum tissue measuring approximately 2x2x15 mm were mounted in 20 ml organ chambers for isometric tension measurement. Each rabbit provided 3-4 strips of corpus cavernosum smooth muscle which were studied separately. The organ chambers contained Krebs-bicarbonate solution composed of (mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, NaHCO₃ 25, MgSO₄ 1.2, KH₂PO₄ 1.2, glucose 11. The solution gassed with 95 % O₂ and 5 % CO₂ during the study and the temperature was maintained at 37°C by a thermoregulated water circuit. One end of each preparation was attached to the bottom of the organ bath while the other end was tied to a force transducer (Grass FT 03) connected to a pen polygraph (Grass 79 E). After mounting, each strip was allowed to equilibrate with a basal tension of 2 g for 1 hour and during this time Krebs-bicarbonate was replaced every 15 min with fresh solution. Two grams of basal tension was chosen for the demonstration of reproducible relaxation to omeprazole. Corpus cavernosum tissue was isolated with an intact endothelium, as assessed by the capacity of acetylcholine (1 μM) to elicit relaxation.

In general, one tissue strip from each rabbit was precontracted with phenylephrine (10 μM) and the second strip was maintained unstimulated. The response of both precontracted strips and strips under basal tension to omeprazole was determined. In some experiments, atropine (1 μM), N⁶-nitro L-arginine methyl ester (L-NAME) (30 μM) or indomethacin (10 μM) was added to the tissue bath during spontaneous contractile activity. After active muscle tone had been induced by phenylephrine (10 μM) or KCl (80 mM), omeprazole (10 μM-1 mM) was cumulatively applied to the bath. After the addition of each dose, we waited until a plateau response was obtained before adding the next one. In some experiments, ammonium chloride (7.5 mM), sodium acetate (7.5 mM), tetraethylammonium chloride (0.5 mM) or glibenclamide (1 μM) was added to the tissue bath after the corpus cavernosum contraction induced by phenylephrine. In some experiments, the nitric oxide synthase inhibitor L-NAME (30 μM) and prostaglandin synthase inhibitor
indomethacin (10 μM) were added in the organ bath 15 min before the
precontraction in order to test the effects of nitric oxide and prostaglandins which
could have contributed to corporal smooth muscle relaxation induced by
omeprazole. In another series of experiment, nimodipine (100 nM-10 μM) was
cumulatively applied to the bath after precontraction with KCl (80 mM).

The following drugs were used in the present experiments: indomethacin (Sigma),
omeprazole (Astra, Södertalje, Sweden), phenylephrine hydrochloride (Sigma),
atropine sulphate (Sigma), L-NAME (Sigma), nimodipine (Sigma), glibenclamide
(Sigma), tetraethylammonium chloride (ICN). All drugs were dissolved in distilled
water except for indomethacin dissolved in 1 % Na₂CO₃, omeprazole dissolved in
dimethyl sulphate. All drugs were freshly prepared on the day of the experiments. In the high K⁺ solution NaCl
was exchanged for equimolar amounts of KCl.

All data are expressed as mean ± the standard error of the mean. In tissues
contracted with phenylephrine or KCl, the relaxant responses were expressed as
percentage of active muscle tone induced by phenylephrine or KCl. Statistical
comparison between groups were performed using general linear models of
analysis of variance (ANOVA) followed by Scheffe's F test. P values of less than
0.05 were considered to be statistically significant.

**Results**

Strips of corpus cavernosum developed spontaneous contractions after they were
mounted in the organ bath. This finding was not evident in all preparations, and its
occurrence varied. Omeprazole decreased and finally abolished all contractile
activity and also decreased resting tone. The effect of omeprazole was slow in
onset and was maximal within 30 minutes. Typical responses are illustrated in
figure 1. However, treatment with atropine (1 μM), L-NAME (30 μM), or
indomethacin (10 μM) did not inhibit spontaneous tone (data not shown).

Omeprazole did not produce any contraction but caused concentration-dependent
relaxation of cavernosal strips precontracted with phenylephrine (Fig 2a, 3). In
order to ensure that relaxation induced by omeprazole was not due to a
nonspecific cytotoxic effect, the reversibility and reproducibility of relaxation
induced by omeprazole was assessed on precontracted corpus cavernosum. In
this process tissues were washed with fresh solution and allowed to recover for
1 h, phenylephrine produced a similar contractile response, suggesting that
relaxation induced by omeprazole is fully reversible. A second application of
omeprazole to the same tissue resulted once more in the complete reversal of
phenylephrine induced tone, suggesting that the maximal response to omeprazole
is fully reproducible (Fig 2b). Omeprazole also relaxed corpus cavernosum smooth
muscle precontracted with KCl (Fig 4).

To investigate whether relaxation induced by omeprazole may be due to an
interaction with the cyclooxygenase or nitric oxide pathways, tissues were
pretreated with indomethacin (10 μM) or L-NAME (30 μM), respectively. Treatment
of cavernosal tissue strips with these synthase inhibitors did not significantly alter
the relaxant response to omeprazole (Fig 3, 4). However, pretreatment with L-
Original tracings showing the effect of omeprazole (0.1 mM to 1 mM) on spontaneous tone of rabbit corpus cavernosum smooth muscle. Representative tracing of original chart record drug of more than 6 individual experiment. All experiments showed similar pattern of response.

Reversibility of omeprazole-induced relaxation of rabbit corpus cavernosum. In a, Omeprazole (0.1 to 1 mM) induces a relaxant response. In b, omeprazole (1 mM) induces a relaxant response and reversed upon washout. After washout and 1 hr recovery period reapplication of phenylephrine causes a similar contractile response and reapplication of omeprazole again causes maximal relaxation. The figure represent an original tracing of a single representative experiment.
NAME increased both the basal tonus and the amplitude of the phenylephrine or KCl contractions recorded in the corporal tissue (data not shown).

In order to determine whether alkalinization or acidification could mediate relaxant responses to omeprazole, the effects of ammonium chloride (7.5 mM) or sodium acetate (7.5 mM) were examined on precontracted tissues. Phenylephrine- or KCl-induced plateau amplitude was not significantly affected by ammonium chloride or sodium acetate (data not shown). Neither ammonium chloride nor sodium acetate had significant effect on relaxant responses to omeprazole (Fig 5).

We investigated the role of potassium channels in the relaxation responses of omeprazole on corporal tissues. We tested responses to omeprazole before and after a 15 min application of 1 μM glibenclamide, an inhibitor of ATP-sensitive potassium channels, or 0.5 mM tetraethylammonium chloride, an inhibitor of calcium-activated potassium channels. Neither glibenclamide nor tetraethylammonium chloride affected the relaxant effect of omeprazole and also they did not cause relaxation. However, tetraethylammonium chloride and glibenclamide increased the amplitude of the phenylephrine contractions recorded in the corporal tissue (Fig 6).

As expected, nimodipine, an L-type calcium channel blocker, relaxed corpus cavernosum precontracted with KCl (Fig 7). Vehicle (polyethenenglycol or dimethyl sulphate) which omeprazole or glibenclamide was dissolved in, respectively, did not have a considerable effect on precontracted corporal strips or on spontaneous contraction (data not shown).
Concentration-response curve of omeprazole in isolated strips of rabbit corpus cavernosum smooth muscle precontracted with KCl alone, in presence of indomethacin and in presence of L-NAME. Each point is expressed as a percentage of the contraction induced by 80 mM KCl and shows the mean (SEM). Number in parentheses indicate the number of preparations used.

Discussion

All spontaneous contractile activity in the corpus cavernosum was blocked upon the addition of omeprazole, but not by indomethacin, L-NAME, or atropine. This finding agrees with findings of Rhoden and his colleagues (2) in guinea pig and human trachea, using omeprazole and suggests that NO, cyclooxygenase products, or acetylcholine do not play a role in the normal spontaneous contractile activity of the corpus cavernosum and spontaneous tone may be involve H⁺-K⁺ ATPase or calcium channel blocking effect of omeprazole. It was demonstrated that removal of extracellular calcium, and addition of calcium antagonists abolished this activity and addition of inhibitors of prostaglandin synthesis markedly reduced spontaneous activity and decreased resting tone and suggested that the spontaneous activity, at least partly, resulted from presence of a stable cyclooxygenase product (5). It has been shown that the spontaneous activity is not affected by tetrodotoxin, atropine, or phentolamine, suggesting a myogenic origin (6). There is no obvious explanation for these variable results.

The results of this study indicate that omeprazole induces relaxation of rabbit corpus cavernosum tissue in vitro at concentrations similar to those found in guinea pig and human airway smooth muscle (2). The human penis has the ability to synthesise various prostanoids and it has been suggested that arachidonic
cascade products may be involved in the control of penile erection (7, 8). The omeprazole-induced relaxation is not mediated by cyclooxygenase products and nitric oxide which is synthesised and released from nonadrenergic noncholinergic nerves and corpus cavernosum endothelium since indomethacin and L-NAME were found ineffective, respectively. According to our knowledge, this result is the first in vitro evidence of relaxant effect of omeprazole in penile tissue smooth muscle. At present the mechanism underlying this relaxation is not known, however two alternate theories can be proposed:

1-Corpus cavernosum relaxation induced by omeprazole is related to its ability to inhibit the H\(^+-\)K\(^+\) ATPase. H\(^+-\)K\(^+\)ATPase inhibition could produce two opposing effects on smooth muscle cells; i)-A decrease in tone due to decreased intracellular pH. It is well known that a number of other smooth muscles are relaxed by a decrease in intracellular pH (4). However this possibility seems unlikely, since in this study the relaxation induced by omeprazole was not changed by alkalinization with 7.5 \(\mu\)M ammonium chloride and by acidification with 7.5 \(\mu\)M sodium acetate. ii)-An increase in tone due to depolarisation which resulted from a decrease in K\(^+\) uptake into cells and physiological increases in extracellular K\(^+\). The depolarisation will stimulate the opening of voltage-dependent calcium channels which increased the tension. This possibility is also unlikely since omeprazole did not cause any contraction.
Monitoring of omeprazole effects on rabbit cavernous smooth muscle precontracted with phenylephrine alone (a), and in presence of tetraethyl ammonium chloride (TEA) (b) or glibenclamide (Gli) (c). Note increase in tone after administration of TEA or glibenclamide. Omeprazole caused complete relaxation (concentrations indicated mM).

2- Corpus cavernosum relaxation is due to some other unspecified property of this compound. The H⁺-K⁺ ATPase has never been described in corpus cavernosum therefore, it is not possible to definitively invoke its inhibition in the mechanism of relaxation. This effect is most probably not related to its H⁺-K⁺ATPase inhibitor action because the concentration of omeprazole required to cause maximal inhibition of H⁺-K⁺ pumps (10⁻⁵ M) (9) is much less than the concentration required for maximal inhibition of contractile responses in the present study (>10⁻⁴ M). However it should be kept in mind that high concentrations of proton pump inhibitors have been reported to affect other ion-motive ATPases likes Na⁺-K⁺ ATPase and vacuolar H⁺-ATPase (10). Omeprazole inhibits responses to phenylephrine and suggests that omeprazole inhibits Ca²⁺ influx through receptor-operated channels. In addition, omeprazole caused relaxation of corpus cavernosum precontracted with KCl and pretreatment of corpus cavernosum with both L-NAME and indomethacin did not blocked the relaxation in response to omeprazole, suggesting that NO-cGMP and prostaglandin pathway may not be involved in this relaxation. The ability of omeprazol to inhibit KCl responses
indicates that omeprazole may inhibit voltage-operated calcium channels, because nimodipine, an L-type calcium channel blocker, also inhibits responses to KCl in rabbit corpus cavernosum smooth muscle suggested that omeprazole and nimodipine inhibit the same calcium channels activated by KCl. It is well known that high K⁺-induced contraction in normal medium is due to an increase in calcium influx through voltage operated channels. Therefore, omeprazole appears to affect activity of at least two different channel types; the mechanism by which this occurs is unknown. The relaxant effect of omeprazole may not be related to the activation of potassium channels, because omeprazole induced dose-dependent relaxation was unaffected by glibenclamide, an inhibitor of ATP sensitive potassium channels and by tetraethyl ammonium chloride, an inhibitor of calcium activated potassium channels.

The H⁺-K⁺ATPase has been identified in vascular smooth muscle (3). A similar compound, NC-1300, has also been found to inhibit acetylcholine induced contractions of the guinea pig ileum, suggesting that benzimidazole derivatives may indeed inhibit smooth muscle tone (11). Omeprazole inhibits the H⁺-K⁺ ATPase through a covalent modification of the enzyme. Omeprazole itself is not active, but requires an H⁺-mediated conversion to a sulphonamide derivative, which then reacts with sulphhydryl groups on specific cysteine residues of the alpha-subunit (12). The requirement of omeprazole to be converted to an active metabolite is consistent with the slower time course of omeprazole-induced relaxation observed in this study.

Omeprazole has been reported to exert other biological effects, such as; the mechanisms of neutrophil chemotaxis, superoxide production and degranulation
(13) and inhibition of cerebrospinal fluid formation (14). None of these effects can shed light on the mechanisms by which H⁺-K⁺ ATPase inhibitors relax the corpus cavernosum. The ability of omeprazole to relax the corpus cavernosum may have a therapeutic significance. Clinically, H⁺-K⁺ATPase inhibitors are used to suppress gastric acid secretion. Omeprazole itself is not likely to be of benefit in this respect in as much as oral administration of doses sufficient to suppress gastric acid secretion in human subjects that yield plasma levels of 1 to 5 μM. Furthermore, the plasma levels are below the level required to relax corpus cavernosum, but intracavernosal administration of omeprazole could be sufficient to relax corpus cavernosum. In addition, it has been reported that during omeprazole treatment in a man painful nocturnal erections developed, without an increase in libido; these erections disappeared when the drug was stopped 6 weeks later (15). Theoretically, omeprazole may prove beneficial in treating erectile dysfunction. It should be combined with other drugs.

In conclusion, this study shows that omeprazole effectively inhibits the spontaneous contraction of isolated rabbit corpus cavernosum and induces relaxation of corporal smooth muscle precontracted. These findings suggest that the function of Ca⁺⁺ channels may be altered by high concentrations of omeprazole. Further work is needed to determine the cellular mechanisms of action by which omeprazole acts on penile smooth muscle.

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References